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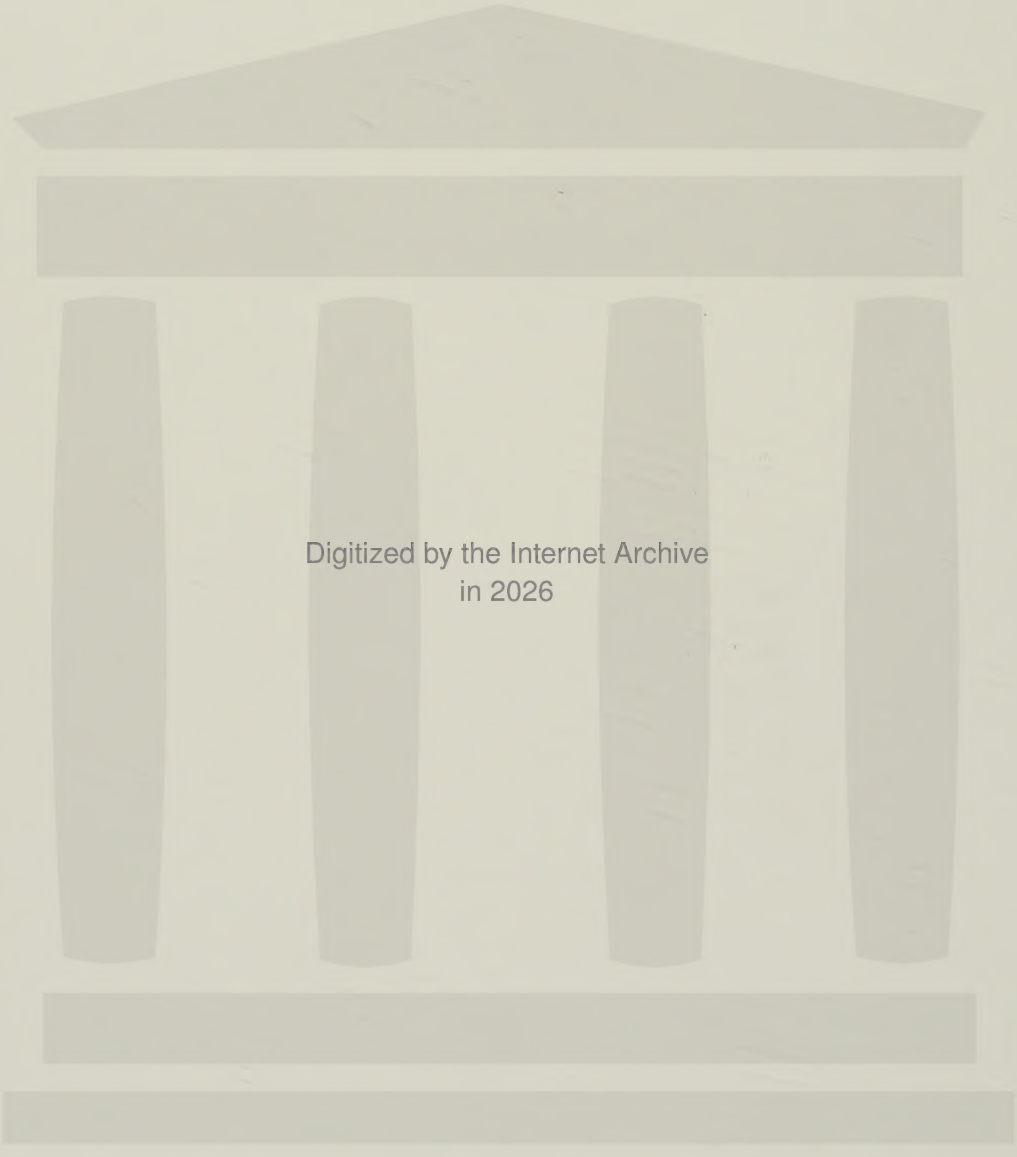

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水産総合研究センター研究報告

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報　文

大容量試料を用いた改良測定法による海水中有機スズ化合物の定量及び海水中での存在状態の考察

隠塚俊満*・田中博之*

Modified analytical method for Organotin Compounds in high-volume water samples, and concentrations in sea water and suspended solids in Hiroshima Bay

Toshimitsu ONDUKA* and Hiroyuki TANAKA*

Abstract To analyze low concentrations of Organotin compounds (OTs) in sea water, we modified a standard analytical method for high-volume water samples. ΣOT concentrations of Hiroshima bay and Kure port measured by this method were 3.2-11ng/L in surface seawater and 1.4-4.7ng/L in suspended solids. Higher concentrations of ΣOTs occurred more as the dissolved form than as the particulate form. The ratios of dissolved forms to particulate ones ranged form 0.51: 0.49 to 0.81: 0.19. Results showed a positive correlation between ΣOT concentration and suspended solids weight.

Key Words: Organotin compounds(OTs), Hiroshima bay, suspended solid(SS), environmental dynamics

有機スズ化合物（OTs）はスズ原子にアルキル基やフェニル基が共有結合する構造を有し，官能基が1個結合するモノ体から4個結合するテトラ体まで多くの化合物から構成される化合物群である。これらのOTsはプラスチックの可塑剤や化学反応の触媒として各種化学工業で使用されるとともに，その殺生物能を利用して農薬や木材防腐剤，船底塗料，漁網防汚剤として使用されてきた（杉田，1992）。OTsの毒性はアルキル基の鎖長がC_4のブチル基で，またトリ体で最も強い。そのため，トリブチルスズ化合物（TBT）が最も多く船底防汚塗料などの殺生物剤として使用された。また，日本では一時期トリフェニルスズ化合物（TPT）も使用された。これらOTs含有船底防汚塗料は防汚効果に優れ，比較的安価で，耐久性があるため広く使用されてきた。また，水流で塗料表面を研磨し，絶えず新しい殺生物能の強い塗料面を露出させる自己研磨型塗料の開発もOTs含有船底防汚塗料の普及に拍車をかけた。

水生生物に関するOTsの毒性試験報告は毒性が強く，かつ広く使用されているTBTに関するものが多い（U.S.EPA., 1996）。貝類の慢性毒性試験では数ng/Lで影響があり（Bryan *et al.*, 1989, Gibbs *et al.*, 1991, Horiguchi *et al.*, 1995），魚類を使った初期生活段階毒性試験では数μg/Lまたはそれ以下の濃度で影響が出ている（Vries *et al.*, 1991; Fent *et al.*, 1992; Triebskorn *et al.*, 1994）。また，一部の巻貝類ではTBT汚染が激しい自然環境中でインポセックスが確認されている（堀口ら，1992；Evans *et al.*, 1995; Smith, 1996; Bech, 2002）。TPTについてはTBTに及ばないものの強い毒性を持つ事が報告されている（小山ら，1992）。

日本ではOTsの一連の海洋環境に及ぼす多大な影響から，業界による自主規制や「化学物質の審査及び製造等の規制に関する法律」（化審法）によって使用の規制が行われ，その結果，海水中における濃度は規制が始まった1990年以降減少し，ここ数年は低濃度

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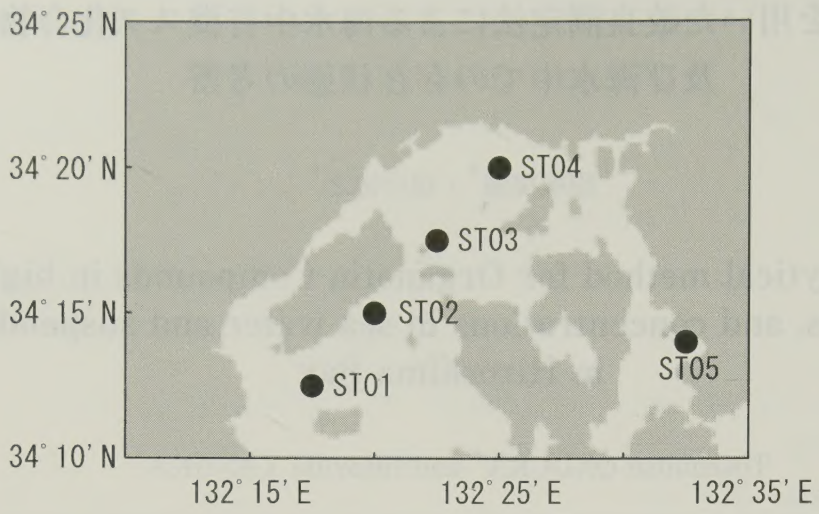

Fig. 1. Sampling location in Hiroshima Bay and Kure Port. Seawater samples were collected from Sts. 1 to 5 (shown as ●)

で横ばい傾向にある（環境省環境保健部環境安全課, 2002）。そのため，TBT, TPT は現在環境省が提案する水試料測定法では検出限界（TBT 3ng/L，TPT 1ng/L）以下の地点が多く，2001年調査における検出頻度は TBT で13/96，TPT で3/96であった（環境省環境保健部環境安全課，2002）。しかし，OTs は内分泌攪乱能を持ち，また非常に低濃度で水生生物に影響がある（Bryan *et al.*, 1988；Khan *et al.*, 1993；Osada *et al.*, 1993）。ちなみに，既報で一番低い影響濃度は TBT 濃度で1ng/L である（Horiguchi *et al.*, 1995）。さらに TPT については TBT と異なり食物網を通し生物濃縮するという報告（池田ら，2002）もあり，低濃度での OTs 動態解明が重要である。

本研究ではより低濃度まで海水中 OTs 濃度を測定するため，環境庁による水質分析法（環境庁環境保健部環境安全課，1998）を以下のように改変し，表層水中 OTs 濃度を測定した。具体的には，分析に供する海水試料を1Lから9Lに増量し，分析に用いる誘導化剤を精製して使用した。また，分析に使用する蒸留水についてもタンクに保存されていた蒸留水ではなく，蒸留装置から直接採取した蒸留水を使用した。一方，浮遊懸濁態物質（SS）中の OTs 濃度については環境庁による底質分析法（環境庁環境保健部環境安全課，1998）を準用した方法で測定し，海水中での OTs の存在状態を考察した。

材料と方法

試料

分析に用いた試料は瀬戸内海区水産研究所所属のしらふじ丸で広島湾及び呉港の ST 1 ～ ST 5 にて採取した（Fig. 1）。海水は表層水をバケツで採取し，表層水をガラス繊維ろ紙（GFC, Whatman）でろ過したものをろ過水とし，ろ紙に捕捉された粒子を SS とした。それぞれろ過水を溶存態，SS を懸濁態として分析に供した。

分析法

1．分析法の概要

環境庁による分析法（環境庁環境保健部環境安全課，1998）を一部改変して OTs を測定した。改変点としては，誘導化剤テトラエチルホウ酸ナトリウム（$NaBEt_4$）を精製して使用したこと，分析に用いる蒸留水を蒸留装置から直接採取した蒸留水を使用したこと，海水中 OTs 分析法にてサンプル量を1Lから9Lへ増量し，それに伴い分析に使用する試薬量を増量したことである。また，SS 中 OTs 分析法では環境省による底質分析法を準用し，SS を吸着したガラス繊維ろ紙ごと分析することにより SS 中 OTs 濃度を測定した。

測定した OTs はモノブチルスズ化合物（MBT），ジブチルスズ化合物（DBT），TBT，モノフェニルスズ化合物（MPT），ジフェニルスズ化合物（DPT），TPT の6種類である。これらの物質は有機スズ化合物イオンに陰性基が配位した物質であるが，今回の測定法では陰性基を塩素イオンで置換して測定した。そのため標準物質としてはモノブチルスズトリクロライド（MBTCl），ジブチルスズジクロライド（DBTCl），トリブチルスズクロライド（TBTCl），モノフェニルスズトリクロライド（MPTCl），ジフェニルスズ

Table 1. Organotin standards

Compound	abbreviation	company	purity（%）
Monobutyltin chloride	MBTCl	Aldrich	95
Dibutyltin dichloride	DBTCl	Tokyo kasei Kogyo	97
Tributyltin chloride	TBTCl	Tokyo Kasei Kogyo	95
Tetrabutyltin	TeBT	Hayashi Pure Chemical	99.2
Monophenyltin trichloride	MPTCl	Albrich	98
Diphenyltin dichloride	DPTCl	Albrich	96
Triphenyltin chloride	TPTCl	Tokyo Kasei Kogyo	98
Tetraphenyltin	TePT	Hayashi Pure Chemical	94.7
Monobutyltin chloride-d_9	MBTCl-d_9	Hayashi Pure Chemical	99.6
Dibutyltin dichloride-d_{18}	DBTCl-d_{18}	Hayashi Pure Chemical	97.2
Tributyltin chloride-d_{27}	TBTCl-d_{27}	Hayashi Pure Chemical	91.5
Monophenyltin trichloride-d_5	MPTCl-d_5	Hayashi Pure Chemical	98.3
Diphenyltin dichloride-d_{10}	DPTCl-d_{10}	Hayashi Pure Chemical	97.2
Triphenyltin chloride-d_{15}	TPTCl-d_{15}	Hayashi Pure Chemical	97.3

ジクロライド（DPTCl），トリフェニルスズクロライド（TPTCl）の混合ヘキサン溶液を使用した。また，OTsはStrem Chemicals社製$NaBEt_4$でエチル化した後分析した。使用したOTs試薬はTable 1にまとめた。

2．試薬の調製

$NaBEt_4$は田尾ら（1999）の方法に準じ精製して使用した。$NaBEt_4$ 10%水溶液を孔径0.2μmの親水性ポリテトラフルオロエチレン（PTFE）メンブレンフィルター（Advantec）でろ過した後，2000rpm，3分遠心分離し上清を得た。溶液の1/10量のヘキサンを添加して2分間振とうし，相分離を行った後ヘキサン層を除いた。この抽出をさらに2回繰り返し，実験に用いた。なお，分析には調製1ヶ月以内の$NaBEt_4$溶液を用いた。サロゲート溶液としてMBTCl-d_9，DBTCl-d_{18}，TBTCl-d_{27}，MPTCl-d_5，DPTCl-d_{10}，TPTCl-d_{15}，濃度それぞれ88.6μg/L，337μg/L，267μg/L，51.1μg/L，168μg/L，413μg/Lの混合ヘキサン溶液を調製した。また，内標準混合溶液としてテトラブチルスズ（TeBT）-d_{36}，テトラフェニルスズ（TePT）-d_{20}，濃度それぞれ813μg/L，1020μg/Lの混合ヘキサン溶液を調製した。

3．海水中OTs分析法

海水中OTs濃度は環境庁による水質分析法（環境庁環境保健部環境安全課，1998）を以下に示すように改変し測定した。分析に供した海水試料を1Lから9Lに増量し，サンプル量の増量に伴って分析に用いる試薬の量を増量した。

具体的な操作としては，10L試料ビンに海水9Lを取り，海水をスターラーで撹拌しながら前記サロゲート溶液20μL（1.77ng MBTCl-d_9，6.74ng DBTCl-d_{18}，5.34ng TBTCl-d_{27}，1.02ng MPTCl-d_5，3.36ng DPTCl-d_{10}，8.25ng TPTCl-d_{15}）を添加した。また，酢酸1.5mL，pH5に調整した2M酢酸－酢酸ナトリウム緩衝液20mL，10%$NaBEt_4$ 1mLを加えた。ヘキサン250mLを加え1時間撹拌後静置し，ヘキサン層を分離後500mL分液ロートに駒込ピペットでヘキサン層を回収した。さらにヘキサン150mLを試料ビンに加え同様に操作し，ヘキサン層を回収し，前のヘキサンと合わせた。分液ロートに無水硫酸ナトリウムを10g加え撹拌して脱水後，濃縮装置（Turbo Vap Ⅱ，Zymark）でヘキサンを約1mLまで濃縮した。ヘキサン2mLでコンディショニングしたフロリジルカラム（Sep-Pak+ florisil，Waters）に濃縮液を添加し，5%ジエチルエーテル－ヘキサン6mLで溶出した。前記内標準溶液20μL（16.3ng TeBT-d_{36}，20.4ng TePT-d_{20}）を添加し，窒素気流下で0.1mLまで濃縮し，GC/MSの試料とした。

4．SS中OTs分析法

SS中OTs濃度は環境庁による底質分析法（環境庁環境保健部環境安全課，1998）を準用した方法で測定した。すなわち，SSをろ過したろ紙をハサミでろ紙ごと細かくしたものを底質とみなし，以下のとおり環境省による底質分析法で測定した。

SSをろ過したろ紙ははさみで細かくし，これを50mLの遠沈管に入れ，前記サロゲート溶液20μL（1.77ng MBTCl-d_9，6.74ng DBTCl-d_{18}，5.34ng TBTCl-d_{27}，1.02ng MPTCl-d_5，3.36ng DPTCl-d_{10}，8.25ng TPTCl-d_{15}）を添加した。1M塩酸－メタノール／酢酸エチル（1:1）混合溶液10mLを加え20分振とう抽出した。抽出液をNo.5Aのろ紙で吸引ろ過し回収した。残さを遠沈管に戻し1M塩酸－メタ

ノール／酢酸エチル（1:1）混合溶液10mLを加え，20分間振とう抽出し再度No.5Aのろ紙で吸引ろ過した。さらに1M塩酸－メタノール／酢酸エチル（1:1）混合溶液5mLで残さを洗浄した。2回のろ過により得られたろ液と洗浄液を合わせた後，約38℃の湯浴上，窒素気流下で酢酸エチル臭がなくなるまで濃縮し，pH5の2M酢酸－酢酸ナトリウム緩衝液20mL，10%$NaBEt_4$ 0.5mLを加え10分間振とうした。ヘキサン5mLで2回振とう抽出してヘキサンを回収し，窒素気流下で約1mLまで濃縮した。2mLのヘキサンで調製したフロリジルカラム（Sep-Pak+ florisil, Waters）に濃縮液を添加し，5%ジエチルエーテル－ヘキサン6mLで溶出した。前記内標準溶液20μL（16.3ng TeBT-d_{36}，20.4ng TePT-d_{20}）を添加し，窒素気流下で0.1mLまで濃縮し，GC/MSの試料とした。

5．検量線用標準試料調製法

標準試料についても分析時毎に誘導体化し，検量線用試料を調製した。25mL試験管に3% NaCl水溶液15mLを取り，標準OTs混合溶液（約1000ng/L）を0－0.2ml添加し，数濃度を調製した。前記サロゲート溶液20μL（8.86ng MBTCl-d_9，33.7ng DBTCl-d_{18}，26.7ng TBTCl-d_{27}，5.11ng MPTCl-d_5，16.8ng DPTCl-d_{10}，41.3ng TPTCl-d_{15}）を添加した。pH5の2M酢酸－酢酸ナトリウム緩衝液0.5mL，10%$NaBEt_4$ 0.3mLを加えた。20分間振とう後ヘキサン3mLを加え10分間振とう後静置し，ヘキサン層を分離後少量濃縮管にヘキサン層を回収した。同様の操作を繰り返し，前のヘキサンに合わせた。前記内標準溶液20μL（81.3ng TeBT-d_{36}，102ng TePT-d_{20}）を添加し，窒素気流下で0.5mLまで濃縮し，GC/MSの試料とした。

6．GC-MS分析条件

キャピラリーカラムとしてDB5-MS（長さ30m，0.25mmID，Agilent Technologies）を装着したGC/MS（HP5890Ⅱ，5972）用い，以下の条件で分析した。注入口の温度は290℃とし，検出器の部分に300℃の温度をかけた。カラム温度は60℃で2分間保持した後，20℃／分で130℃まで昇温，引き続き10℃／分で210℃まで昇温後，5℃／分で300℃まで昇温し，2分間保持のプログラムによった。1検体に要する分析時間は30分であった。SIMモードでMBT，DBT，TBT，MPT，DPT，TPTとそれぞれのd体，TeBT-d_{36}，TePT-d_{20}を定量した。モニターイオン，確認イオンはそれぞれm/zでMBT：235，233，DBT：261，263，TBT：263，261，MPT：253，255，DPT: 303，301，TPT：313，301，MBTCl-d_9：244，242，DBTCl-d_{18}：279，281，TBTCl-d_{27}：318，316，MPTCl-d_5：260，258，DPTCl-d_{10}：313，311，TPTCl-d_{15}：366，364，TeBT-d_{36}：318，316，TePT-d_{20}：366，364とした。内標準物質でOTsとd体の定量値を補正後，OTsそれぞれに対応するd体の回収率で定量値を補正し，標準試料を基準にして検量線を作成し濃度を計算した。

結果と考察

分析法の改変

OTsのクロマトグラムをFig.2に示す。海水中OTsを感度良く分析するために，環境省による分析法を基に，分析試料の量を多くすることを検討した。環境省による分析法では1Lの水を分析に供するが，これを9Lにして分析した。しかし，サンプル量を多くするとOTsの値だけでなくblank値も高くなり，定量値の信頼性が低いものになってしまった。特にMPTでは妨害ピークが存在し，濃度が低いサンプルは適切に定量できなかった（Fig.3 a, b）。

しかし，田尾らの方法により誘導化剤を精製して利用することにより，blankの低下，妨害ピークの減少が見られた（Fig.3 c, d）。また，DPTなどのOTsはプラスチックの可塑剤に利用されているため，その影響を考慮して分析に使用する蒸留水はタンクに保存されたものではなく，蒸留装置から直接採取して利用した。

このような分析法の改変の結果，OTsを添加していない0ng/L標準試料のクロマトグラフ（Fig.4）から得られたアバンダンスを元に，海水およびSSについて試料9Lとして計算するとS/N比が3に相当する濃度は海水でそれぞれMBT 0.1ng/L，DBT 0.05ng/L，TBT 0.06ng/L，MPT 0.5ng/L，DPT 0.01ng/L，TPT 0.01ng/Lとなった。

海水中におけるOTsの存在状態

表層水，ろ過水，SS中有機スズ化合物濃度をTable.2に示した。ろ過水とSS中のOTs濃度を合計するとほぼ表層水の濃度となった（Table 2 a, b, c）。しかし，時期，地点により，ろ過水の濃度が表層水の濃度と同等または超える状態が見られた（2002 Oct. ST02等）。また，ろ過水とSS中のOTs濃度を表層水のOTs濃度で割ることにより溶存態中および懸濁態中OTsの存在割合を計算した（Table 2 d, e）。

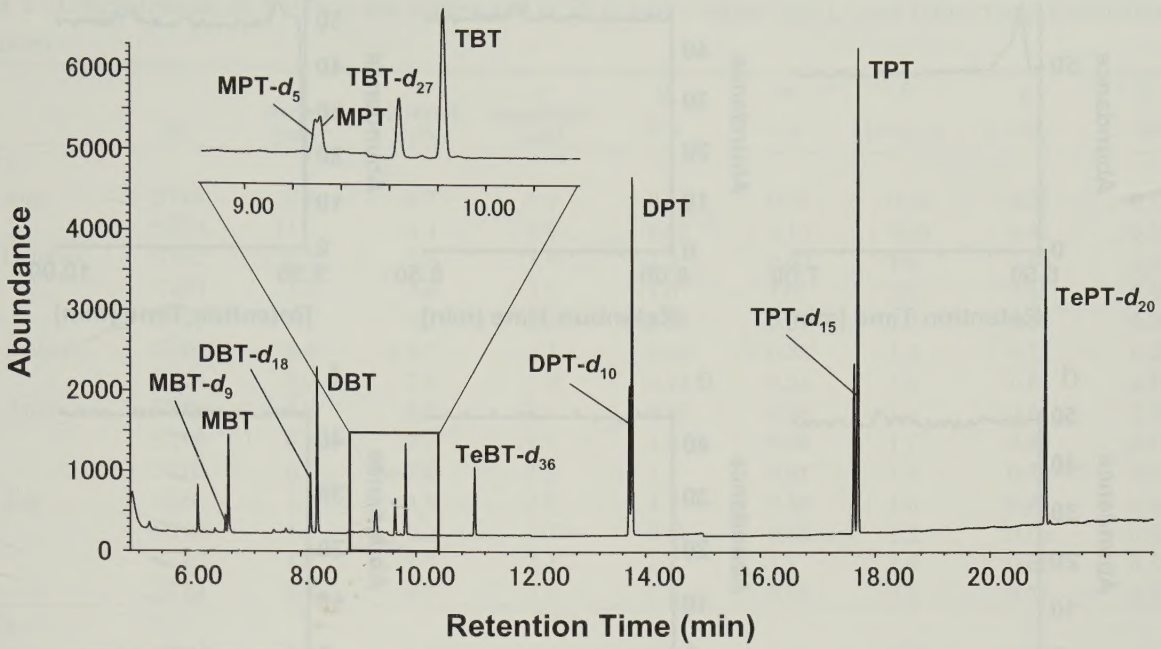

Fig. 2. GC-MS choromatograms of OTs standard solution

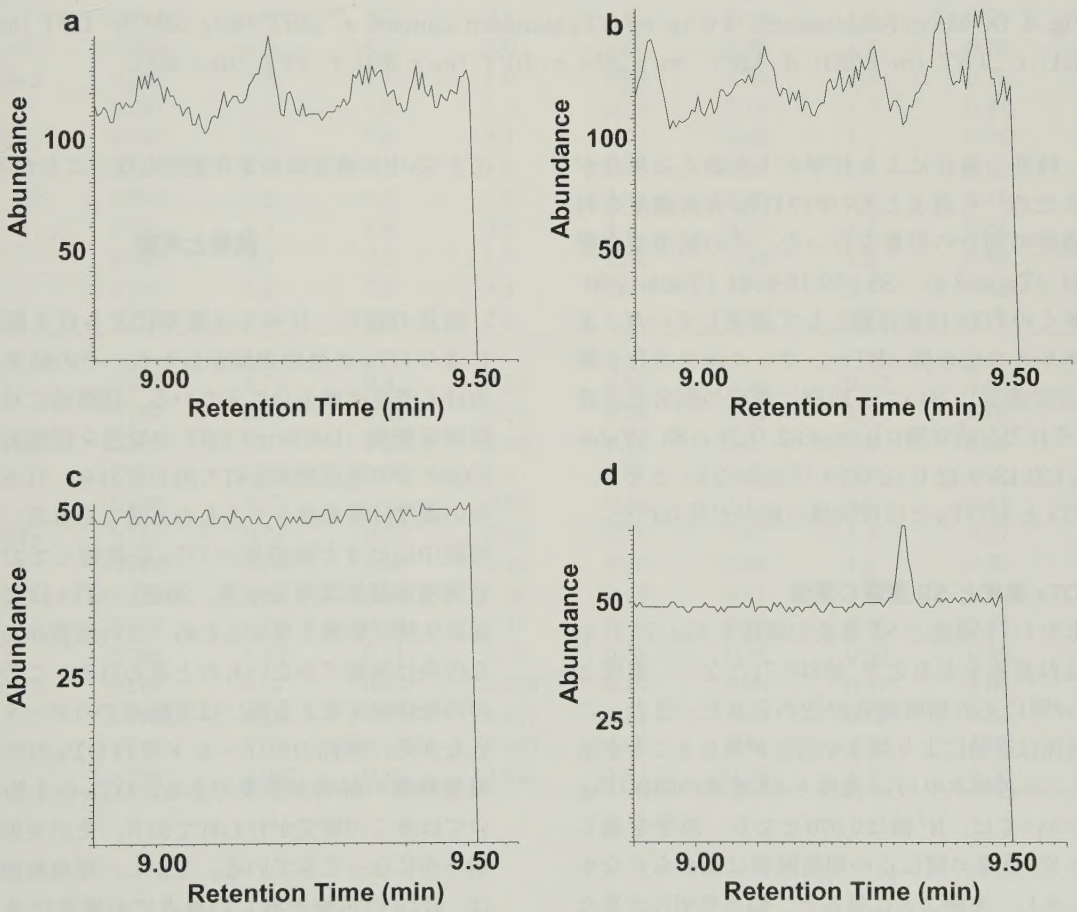

Fig. 3. GC-MS (m/z 253) chromatograms of 0ng/ml (a, c) and 50ng/L (b. d), MTP standard samples. Chromatograms of MPT standard samples ethylated by non-refined $NaBEt_4$ and those ethylated by refined $NaBEt_4$ were a, c and b, d, respectively.

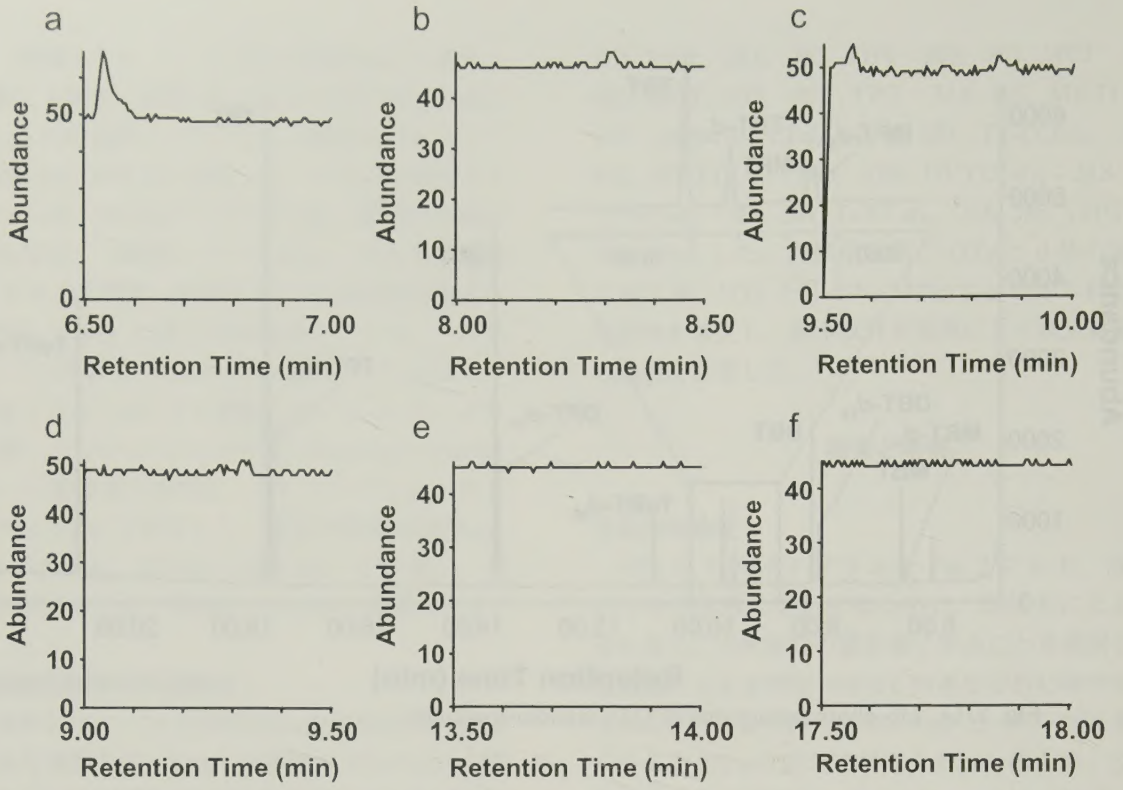

Fig. 4. GC-MS chromatograms of 0 ng/ml OTs standard samples. a : MBT (m/z 235). b : DBT (m/z 261). c : TBT (m/z 263). d : MPT (m/z 255). e : DPT (m/z 303). f : TPT (m/z 351).

しかし，時期，地点により比率が1を超える場合が出現するため，ろ過水とSS中のOTs合計濃度を利用して濃度の割合の計算を行った。その結果溶存態0.51-0.81（Table 2 g），SSに0.19-0.49（Table 2 h）となり多くのOTsは溶存態として存在していた。また，ブチルスズ化合物（BTs），フェニルスズ化合物（PTs）合計濃度においても同様に濃度の割合を計算すると，それぞれ溶存態に0.58-0.82, 0.21-0.88（Table 2 g），SSに0.18-0.42, 0.12-0.79（Table 2 h）となり，BTs，PTsともOTsとほぼ同様の傾向が見られた。

海水中OTs濃度とSS重量の関係

表層水中OTs濃度とSS重量の関係をFig. 5 aに示した。近似直線をとるとR^2値は0.71となり，濃度とSS重量の間に正の相関関係が認められた。また，この相関関係は季節により傾きや切片が異なることが示唆された。ろ過海水中OTs濃度とSS重量の関係（Fig. 5 b）については，R^2値は0.070となり，四季を通じて濃度とSS重量の間に正の相関関係は認められなかった。しかし，季節ごとに見ると，傾きや切片が異なるものの，濃度とSS重量の間に正の相関関係が示唆された。一方，SS中OTs濃度とSS重量の関係（Fig. 5 c）を見ると，例外はあるもののSS重量が変化してもSS中の濃度はあまり変化しないことが示された。

結果と考察

前述の通り，日本では業界による自主規制や法律によりOTsの使用規制が行われ，その結果海水中における濃度は減少してきている。国際的に見ても，国際海事機関（IMO）がTBTの使用を段階的に規制し2008年での使用禁止を打ち出しており，日本同様海水中の濃度は将来減少するものと考えられる。しかし，底泥中にはまだ高濃度のOTsが残留しており（環境省環境保健部環境安全課，2002），OTsはごく微量で海洋生物に影響を与えるため，この底質からの二次的な汚染は無視できないものと思われる。この底質からの汚染状況を考える際には実験系でのデータもさることながら，実際のフィールドでのOTsの生物濃縮と環境動態の解明が重要である。OTsの生物濃縮については多くの研究が行われており，その実態がかなり明らかになってきている。しかし，環境動態に関しては，OTsの汚染が激しい地点での研究は多く存在するが，水系への汚濁負荷が少ない環境で底質からの汚染が考えられる地点において，OTsの動態を解明した研究は皆無である。また，この種の研究を行う際に

Table 2 . Concentrations of ΣOTs in sea waters and in SS in four seasons (ng/L), and proportional environmental dynamics

	No.	a surface water	b filtered water	c suspendid solid	d b/a	e c/a	f (b+c)/a	g b/(b+c)	h c/(b+c)
ΣOTs									
2002 July	ST02	9.4	5.7	1.4	0.61	0.15	0.75	0.81	0.19
	ST04	11	6.4	1.5	0.57	0.13	0.70	0.81	0.19
2002 October	ST02	3.2	3.8	1.9	1.2	0.60	1.8	0.66	0.34
	ST04	4.7	4.8	4.7	1.0	1.0	2.0	0.51	0.49
	ST05	7.1	6.7	2.6	0.95	0.37	1.3	0.72	0.28
2003 January	ST02	5.6	5.3	2.1	0.95	0.38	1.3	0.71	0.29
	ST04	8.0	7.5	1.8	0.94	0.22	1.2	0.81	0.19
2003 April	ST02	6.2	4.2	2.4	0.68	0.38	1.1	0.64	0.36
	ST04	6.8	9.5	1.7	1.4	0.26	1.7	0.85	0.15
	ST05	6.1	7.0	1.9	1.1	0.31	1.5	0.79	0.21
2003 July	ST01	4.7	4.9	2.5	1.1	0.53	1.6	0.66	0.34
	ST02	4.1	3.3	1.7	0.81	0.42	1.2	0.66	0.34
	ST03	4.2	7.4	4.4	1.7	1.1	2.8	0.62	0.38
	ST04	5.4	9.1	2.8	1.7	0.51	2.2	0.77	0.23
ΣBTs									
2002 July	ST02	8.7	5.3	1.1	0.61	0.13	0.74	0.82	0.18
	ST04	11	6.0	1.3	0.56	0.12	0.67	0.82	0.18
2002 October	ST02	3.0	3.6	1.4	1.2	0.47	1.7	0.72	0.28
	ST04	4.5	4.5	3.3	1.0	0.74	1.7	0.58	0.42
	ST05	6.5	6.2	2.0	0.96	0.30	1.3	0.76	0.24
2003 January	ST02	4.8	4.8	1.6	0.99	0.34	1.3	0.75	0.25
	ST04	7.2	6.9	1.3	0.96	0.18	1.1	0.84	0.16
2003 April	ST02	5.4	3.8	2.1	0.71	0.39	1.1	0.65	0.35
	ST04	6.0	9.1	1.6	1.5	0.26	1.8	0.85	0.15
	ST05	5.2	6.5	1.5	1.3	0.30	1.6	0.81	0.19
2003 July	ST01	4.4	4.9	2.5	1.1	0.56	1.7	0.66	0.34
	ST02	3.9	3.2	1.7	0.82	0.43	1.3	0.65	0.35
	ST03	4.2	7.3	4.4	1.7	1.1	2.8	0.62	0.38
	ST04	5.4	9.0	2.8	1.7	0.51	2.2	0.76	0.24
ΣPTs									
2002 July	ST02	0.70	0.41	0.24	0.58	0.34	0.92	0.63	0.37
	ST04	0.54	0.45	0.24	0.84	0.44	1.3	0.66	0.34
2002 October	ST02	0.27	0.21	0.55	0.76	2.0	2.8	0.28	0.72
	ST04	0.29	0.38	1.5	1.3	5.1	6.4	0.21	0.79
	ST05	0.57	0.49	0.63	0.86	1.1	2.0	0.44	0.56
2003 January	ST02	0.74	0.52	0.50	0.71	0.67	1.4	0.51	0.49
	ST04	0.75	0.57	0.46	0.76	0.62	1.4	0.55	0.45
2003 April	ST02	0.86	0.38	0.26	0.44	0.31	0.74	0.59	0.41
	ST04	0.78	0.46	0.17	0.59	0.21	0.80	0.73	0.27
	ST05	0.92	0.51	0.35	0.55	0.38	0.93	0.59	0.41
2003 July	ST01	0.27	0.031	0.016	0.11	0.06	0.17	0.66	0.34
	ST02	0.16	0.083	0.021	0.53	0.14	0.67	0.80	0.20
	ST03	0.036	0.035	0.033	0.98	0.94	1.9	0.51	0.49
	ST04	0.033	0.13	0.018	3.8	0.54	4.4	0.88	0.12

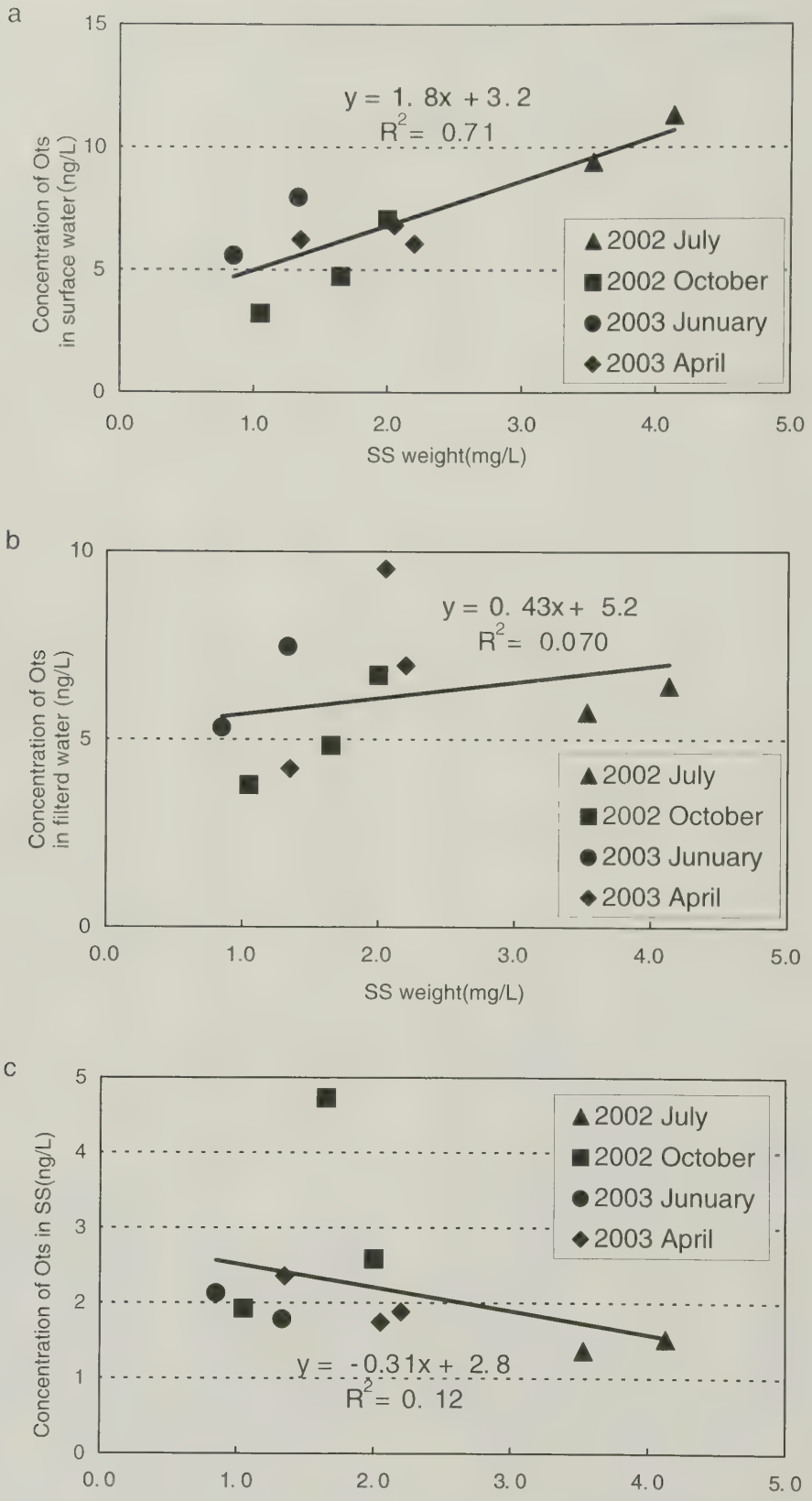

Fig. 5. Relationship between SOTs concentration and SS weight. a : SOTs concentration in surface water versus SS weight. b : SOTs concentration in filtered water versus SS weight. c : SOTs concentration in SS versus SS weight.

は従来の方法では検出限界以下である濃度においても精度良くOTs濃度を測定する必要があり，分析法の改良を行う必要があった。

今回の測定法では既法よりサンプル量を10倍程度増やしたため，OTsを10倍程度感度良く分析が可能になることが期待された。海水試料9Lとするとと S/N比が3に相当する濃度は海水でそれぞれMBT 0.1ng/L，DBT 0.05ng/L，TBT 0.06ng/L，MPT 0.5ng/L，DPT 0.01ng/L，TPT 0.01ng/Lとなった。一方環境省による分析法では検出限界がそれぞれDBT 0.42ng/L，TBT 0.31ng/L，MPT 0.55ng/L，DPT 0.25ng/L，TPT 0.17ng/Lである。S/N比と検出限界では数値の計算方法が異なるため一概には比較できないが，MPTなど物質によっては測定感度がほとんど変わらないものも存在した。今回サンプル量を10倍にすることにより，毒性の強いTBTやTPTの測定感度を向上させることができた。

今回の海水中OTs濃度測定では，時期，地点によってはろ過水とSS中の濃度の合計が表層水中の濃度を超える状態が見られた（Table 2）。この原因としては，低濃度での測定による誤差の上昇や含まれるSS中のOTsの誘導体化が不十分である可能性が考えられた。しかし，ろ過水中とSS中の合計値は大きい場合でも表層水のOTs濃度の2倍程度であり，非常な低濃度の測定値であることを考慮すると特に悪い精度ではないと考えられる。

本研究の結果では，海水中OTsの多くは溶存態として存在しており，海水中OTsが懸濁態として存在するという過去の報告（Randall *et al.*, 1986）と相反する結論となった。しかし，最近の瀬戸内海での報告（田尾ら，1999）では海水中ではBTs，PTsの多くは溶存態として存在しており，本研究の結果と一致している。過去の報告は汚染の激しい時期，地点の報告がほとんどであり，今回のような条件では有機スズ化合物の挙動が異なることが推測された。

また，表層水中OTs濃度とSS重量の間には正の相関が認められた（Fig. 5 a）。また，季節ごとに見ていくと季節により傾きや切片が異なる事が示された。ろ過海水中OTs濃度とSS重量の関係（Fig. 5 b）においては，年間を通して正の相関関係は示されなかった。しかし，季節ごとに見ると，季節により傾きや切片が異なるものの，OTs濃度とSS重量の間に正の相関関係が存在した。そのため，海水中OTs濃度はSS重量のみに依存して濃度が変化するわけではないことが示唆された。つまり，海水中の懸濁態OTs濃度はSS濃度に依存して高くなり，溶存態OTs濃度はSS濃度が大きくなる環境中で高くなると考えられた。この2つの要因が重なることにより，SS重量が大きくなると海水中OTs濃度が高くなると推測された。

OTsの存在状態やOTs濃度のSS重量との関係について全体の傾向から外れる地点，時期が存在するが，これらのほとんどがST 4のデータである。ST 4は太田川の河口に近く，河川水の影響を受けやすい地点であるため，河川水の影響についても考慮する必要がある。

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Bull. Fish. Res. Agen. No. 13, 11-14, 2004

短　報

Where's the cadmium in biogenic particulate matter?

Kazuo ABE*

Abstract A preliminary study on the cadmium (Cd) distribution in biogenic particulate matter was carried out in the subtropical sea near Ishigaki Island. Almost all of the biogenic Cd was determined to be associated with the organic materials, and the possibility of the estimation of the Cd adsorbed onto the surface of organic materials and associated with metalloproteins was suggested; however further methodological examination such as checking the dissolution of the calcium carbonate ($CaCO_3$) phases (hard protective shell) during the chemical leaching treatment of acid hydrogen peroxide (H_2O_2) might be necessary.

Key Words: cadmium, biogenic particulate matter, chemical leaching treatment, subtropical sea

Many reports on the good correlation of cadmium (Cd) behavior with marine biogeochemical processes have been published in the last two decades (e.g., de Baar *et al.*, 1994), suggesting an important roll of marine biota such as phytoplankton for the Cd carrier regulating the distribution in the ocean; however, which part in the planktonic organisms (such as calcium carbonate ($CaCO_3$) phases (hard protective shell) and organic materials) is an efficient carrier has not been clearly elucidated. The objective of this short paper is to report a preliminary study on the distribution of Cd in biologically produced particulate matter, which is considered to be the first step for the topic noted above.

Surface water samples were taken at five stations on the 123° E line (St. 1: 24° 00′ N; St. 2: 23° 30′ N; St. 3: 23° 00′ N; St. 4: 22° 30′ N; St. 5: 22° 00′ N) in the subtropical sea near Ishigaki Island using an acid-cleaned plastic bucket aboard the R/V Yoko-Maru of the Seikai National Fisheries Research Institute (SNFRI) on March 9 and 10, 2003 (YK02-14) (Fig. 1). The samples for particulate Cd were collected by filtration (8.75 L) through a 0.6-μm Nuclepore filter (Corning) on board, and the filters were frozen immediately at -40℃. In this study, biogenic particulate Cd was defined as two fractions for the convenience's sake, i.e.; acetic acid

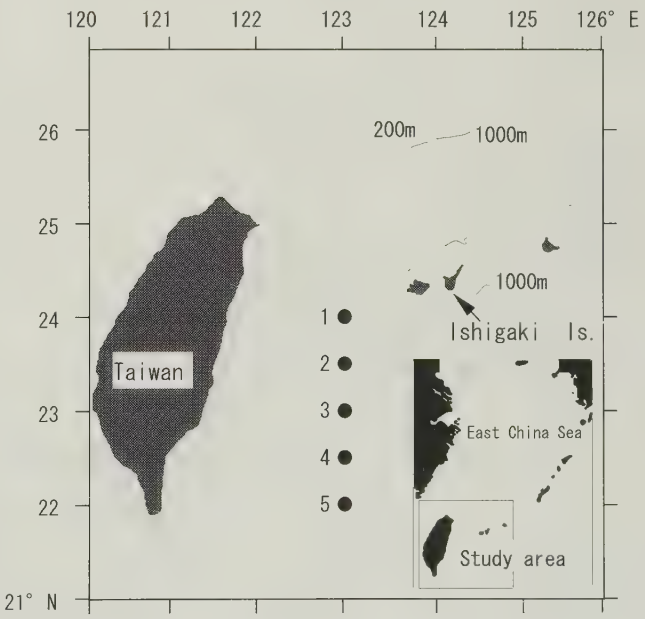

Fig. 1. : Sampling sites in this study

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(HAc) leachable and hydrogen peroxide (H_2O_2) soluble fractions. In general, these fractions are considered to be adsorbed cations, carbonate phases, and associated with metalloproteins (HAc) (Collier and Edmond, 1984; Löscher *et al.*, 1998), and the Cd in organic materials (H_2O_2), respectively (Kitano *et al.*, 1980; Noriki *et al.*, 1997). Usually, in the chemical leaching experiment using HAc and H_2O_2, HAc leaching has been conducted prior to H_2O_2 treatment. However, this procedure can't discriminate the Cd in the HAc leachable organic materials from the Cd associated with $CaCO_3$ (adsorbed and incorporated). So, in this study, determination of Cd was examined by two procedures as follows (Fig. 2):

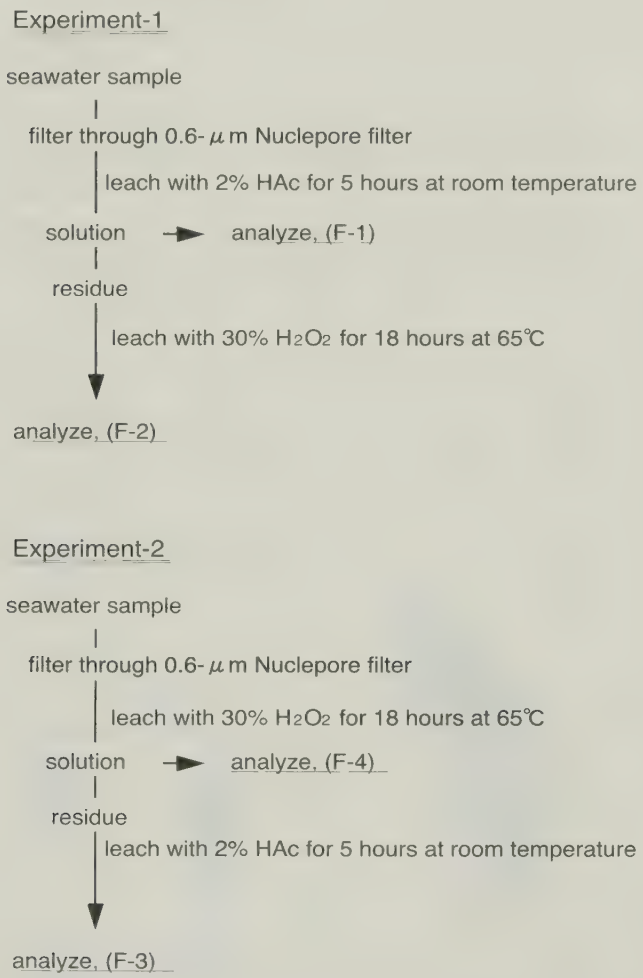

Fig. 2. : Flow diagram of selective chemical leaching techniques

1) Experiment-1 (Ex.-1): Particulate Cd on a 0.6-μm Nuclepore filter was put into 2 mL of 2% HAc solution for 5 hours at room temperature, and the solution was filtered through a 0.4-μm Nuclepore filter(F-1). The residue on the filter was transferred into a Teflon vessel; 2 mL of 30% H_2O_2 solution was added and kept at 65℃ for 18 hours (F-2). This procedure was carried out for samples from all stations.

2) Experiment-2 (Ex.-2): The order of chemical leaching in Ex.-1 was changed; namely 30% H_2O_2 treatment (F-4) was carried out prior to 2% HAc leaching (F-3). This procedure was carried out for only one sample from St. 5. The determinable Cd in each fraction is shown in Table 1, and combining these two procedures made it possible to determine the Cd in the HAc leachable organic phases, namely adsorbed onto the surface of organic materials and associated with metalloproteins (difference between (F-1) and (F-3), and (F-4) and (F-2)). All the procedures for Cd analysis were conducted in a laminar flow bench, and the Cd in these fractions was determined by atomic absorption spectrophotometry (using a Hitachi Z-8270 flameless atomic absorption spectrophotometer(AAS)). A standard addition method was adopted for the determination to correct for matrix interference, and the standard solution was prepared from a commercially available standard solution (1000 ppm, Wako Chemicals). The grade of used chemical regents, HAc and H_2O_2 were the S. S. G and S (Wako Chemicals), respectively. Blank values (Cd) of the procedure were 0.009 pM and 0.012 pM for HAc and H_2O_2 treatment, respectively.

Seawater analysis data were listed in Table 2. Salinity (using a Tsurumi Seiki Degi-Auto 3-G salininometer) and temperature (thermometer on board) were in the range 34.87-34.97 and 22.8-25.2℃, respectively. Phosphate (PO_4) and dissolved Cd (by colorimetry and AAS described in Abe (2002)) were almost depleted, and in regard of these parameters, it is considered to be no great differences in the oceanographic conditions among the sampling sites in this study. Although a microscopic observation of the collected particulate matter was not carried out in this study, relative abundance of the surface phytoplankton near this study sites (22°30′ N; 124°40′ E) have been reported as 54.6, 38.5, and 6.9 % for dinoflagellates, haptophyceae, and diatom, respectively (SNFRI,

Table 1. Determinable Cd in each fraction

Ex.-1 : HAc	(F-1)	①	②	③	④	
Ex.-1 : H_2O_2	(F-2)					⑤
Ex.-2 : HAc	(F-3)	①	②			
Ex.-2 : H_2O_2	(F-4)			③	④	⑤

*① : absorbed onto the surface of $CaCO_3$ phases
*② : incorporated into the $CaCO_3$ phases
*③ : absorbed onto the surface of organic materials
*④ : assosiated with metalloprotein
*⑤ : in the rest of the organic materials

2000). Table 3 shows the concentrations of Cd and the percentages to the determined total biogenic Cd (in the parentheses) in each fraction((F-1), (F-2), (F-3), and (F-4)). From Ex.-1, the concentrations of particulate Cd in the (F-1) and (F-2) fractions varied from 0.40 to 0.59 pM and from 0.072 to 0.13 pM, respectively. From Ex.-2, (F-3) and (F-4) fractions were 0.015 pM and 0.49 pM, respectively, which suggested that almost all (97%) of Cd was in the organic fraction. Furthermore, by subtracting (F-3) from (F-1, average) and (F-2, average) from (F-4), much similar Cd concentrations were calculated as 0.46 and 0.39 pM, respectively, showing the possibility of the estimation of the Cd adsorbed onto the surface of organic materials and associated with metalloproteins using the combination of two methods described in this study although only one result for (F-3) and (F-4) fractions has been obtained from St. 5. However, the possibility of part of the $CaCO_3$ dissolution into the acid H_2O_2 solution (pH; around 4.0) under heating treatment at 65℃ for 18 hours could not be eliminated (which could lead to the overestimation of the determined (F-4) fraction), and checking this dissolution is considered to be a problem to be solved in future. Thus, further methodological examination such as the electron microscopic observation of the $CaCO_3$ dissolution during the chemical leaching treatment

Table 2. Seawater analysis results

	Temp. (℃)	Salinity	PO_4 (μM)	dissolved Cd (nM)
St. 1 (0m)	24.0	34.97	nd	nd
St. 2 (0m)	22.8	34.95	nd	nd
St. 3 (0m)	22.9	34.96	nd	0.007
St. 4 (0m)	25.2	34.87	0.08	nd
St. 5 (0m)	24.3	34.97	nd	0.008

* Less than 0.05 μM of PO_4, and 0.007 nM of Cd(2.5 times of the deviation of the blank)are shown as "nd".

Table 3. Concentration of Cd in each fraction. Percentages are shown in parentheses.

	(F-1) (pM, (%))	(F-2) (pM, (%))	(F-3) (pM, (%))	(F-4) (pM, (%))
St. 1 (0m)	0.40 (75)	0.13 (25)		
St. 2 (0m)	0.59 (84)	0.11 (16)		
St. 3 (0m)	0.43 (80)	0.11 (20)		
St. 4 (0m)	0.43 (86)	0.072 (14)		
St. 5 (0m)	0.52 (85)	0.089 (15)	0.015 (3)	0.49 (97)
average	0.47 (82)	0.10 (18)		

of H_2O_2 might be necessary.

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技術報告

太平洋戦争以前および終戦直後の日本のまぐろ漁業データの探索

岡本浩明*

Search for the Japanese tuna fishing data before and just after World War II

Hiroaki OKAMOTO*

Abstract Existing Japanese tuna fishery data before and just after the World War II was investigated and summarized in this paper. As the reliable statistics and fishing data before the war, "Annual statistics of Ministry of agriculture and forestry" and the data of longline and pole and line operations made by prefectural research vessels were recognized to be available.

In the former Japanese official statistics, fishing vessel statistics categorized by fishing gear and annual catch statistics for tunas and relevant fish groups had been compiled since 1905 and 1894 (1922 for billfishes), respectively. The annual catch of skipjack tuna was 30,000 - 50,000 MT before 1914 when coastal fisheries were main, increased to 60,000 - 80,000 in 1915 - 1935, and up to more than 100,000 MT in 1936 - 1940 because of the development of offshore pole and line fishery. Total catch of tunas, which was less than 20,000 MT before 1918, had increased steeply according as the development of the offshore longline and driftnet fisheries derived from the motorization of the fishing vessel and it exceeded 60,000 MT in 1929.

The fishing data of the prefectural research vessels was recorded for 10 years from 1933 to 1942, and total number of available longline and pole and line operations were 5,302 and 3,315, respectively. Main fishing ground of their longline operation from 1934 to 1937 was distributed at north-western Pacific Ocean north of 20°N targeting albacore. After 1938, their fishing ground had extended and shifted toward tropical region of western Pacific from 20° N to the equator targeting mainly yellowfin tuna. The fishing operation data recorded indicates that the night-setting longline operation, which is used to catch swordfish or sharks effectively, was already common before the war. Usefulness of these fishery data for the stock assessment is not clear and would be different for each species. It ought to be cleared through the analyses comparing pre- and post-war data.

Just after the finish of the war, the fishing data of commercial longline and pole and line fisheries were started to be collected by transcribing logbooks at the major landing port. However, the data from 1946 to 1951 has been remained as the hand writing data sheet to be compiled.

Key Word: pre-war, Japanese tuna fisheries, statistics, research vessel, fishing data

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1. はじめに

インド洋のIOTC（インド洋まぐろ委員会），大西洋のICCAT（大西洋まぐろ類保存委員会），東部太平洋のIATTC（全米熱帯まぐろ委員会），北太平洋のISC（北太平洋まぐろ類暫定科学者委員会），中西部太平洋のSCTB（まぐろ・かじき常設委員会）等の国際漁業管理および資源評価機関のもと，各関係諸国の研究者が参集し，まぐろ類，かつお，かじき類などの資源評価が年々行われている。解析の手法は様々であるが，対象資源の資源量（豊度）変化を簡便に観察する方法として，標準化したCPUE（単位努力量あたりの漁獲量），特にはえ縄のCPUEがよく用いられる。

日本のはえ縄漁業は世界に先駆けて1952年から遠洋域に進出し，1960年代前半にはすべての大洋の主だった漁場ですでに操業を行っていた（上村，1966）ことに加え，そのデータの信頼性の高さから，その操業・漁獲データはそれら資源評価にとって欠くべからざるものとなっている。後述するように，戦後の漁業水域の制限（マッカーサー・ライン）が1952年に撤廃された後に日本の遠洋まぐろ漁業が本格的に始まったため，1952年以降の漁獲データが編纂され，資源解析にも用いられているわけであるが，それ以前，特に太平洋戦争以前にかつお・まぐろ漁業が無かったわけではない。

2003年5月にイギリスのNature誌に掲載されたMyers and Worm（2003）は，戦後に遠洋漁業が始まった後，外洋性大型魚類の処女資源は急激に減少したとする説をそれらのCPUE（単位努力量あたりの漁獲量）の変化から主張したものであり，日本をはじめ世界の資源研究者の間に物議を醸した。多くのまぐろ漁業の主漁場では，太平洋戦争以前にも戦後には遠く及ばないものの，すでにかなりの漁獲があった。それらをなるべく過去に遡って解析し，処女資源からの資源状態の変動を正確に把握する必要性が認識され始めたものの，少なくとも資源解析に用いるデータセットには戦前および戦後直後のデータとしては存在しない。

そのような背景のもと，ハワイ大学が中心となっているPFRP（Pelagic Fisheries Research Program：外洋漁業調査計画）のワークショップの開催が予定されており，そのテーマが「Data Rescue: 過去のデータの掘り起こし」であり，日本の戦前のかつお・まぐろ漁業データの現状について日本の研究者に紹介してほしい，とのリクエストが寄せられた。ちょうどそのリクエストを受けとる直前に，大正7年くらいから発行されている「海洋調査要報」という定期（半年に1冊）刊行物に昭和7年頃から太平洋戦争開始まで，各道府県（当時，東京都は東京府であった）の水産試験場調査船の操業ごとの漁獲データが収集されていることを知り，データとしての利用可能性を検討し始めていた。そこで，利用しうる戦前および終戦直後のデータをさらに探索・整理した上で，このワークショップに出席した。本報告は，2003年12月9日～11日にハワイ大学構内で開催されたPFRPワークショップにおける著者の発表内容を本誌用にまとめたものである。

なお，本文に登場するかつお・まぐろ漁業に関する歴史的背景の記述の多くは，「かつお・まぐろ総覧」（かつお・まぐろ総覧編纂委員会編，1963）および「焼津水産史　上巻」（焼津水産史編纂委員会編，1981）から引用・参照した。また，本報で使用した統計資料についてはAppendix Table 1に一覧とした。

2. 太平洋戦争以前のかつお・まぐろ類漁獲データ

1) 農林統計

通称「農林統計」とは，今日の農林水産省経済局統計情報部によって編纂されている日本の農業，林業，水産業等にかかわる公的な基本統計であり，その歴史は明治元年（1867年）にまで遡るが，水産業の漁獲物に関する統計が加わったのは若干遅れて明治27年（1894年）である。漁船数についてもサイズ別，船型別の統計が明治27年（1894年）から編纂されており，明治38年（1905年）からは漁業種類別漁船数が加わった。編纂された統計は基本的には該当年の翌年もしくは翌々年に農林水産省統計表として本にまとめられるが，この名前は時代によって農商務統計表（1884年～1923年），農林省統計表（1924年～1941年），農商省統計表（1942年～1943年），農林省統計表（1944年～1977年），農林水産省統計表（1978年～現在）と変化してきている。

各年の統計表には，該当年のたとえば県別，漁業種類別といった詳細な統計値と，比較のために前数年間の各年合計値が記載されているが，長年にわたる値を一覧するには多くの分厚い統計表をめくらねばならない。そこで，昭和7年から昭和30年の間に5種類の漁獲累年統計表が刊行されており，また，水産統計のみを扱った「海面漁業，養殖業生産年報」の昭和36，43～51年度版には大正元年から各最新統計年までの，そして昭和52～54，59～平成13年度版には昭和元年から各最新統計年まで更新した漁獲累年統計が「参考表」として掲載されている。しかし，累年統計表は毎年発

行される統計表のように魚種別漁獲量が漁業種類別になっておらず年計値であるというように，荒い分類での集計値しか掲載されていないのが難点である。ここでは，なるべく累年ではなく各年の統計表を参照し，主にかつお・まぐろ漁業にかかわる漁船数および水揚量の統計を紹介する。また，漁船数および水揚量のいずれにおいても，本文では全国の値を合計した年計値を用いるが，各年の統計表には県別の集計値も記載されている。

また，太平洋戦争以前には，日本は占領国であり，日本本土，いわゆる内地に対して，露領，関東州，朝鮮，台湾，南洋諸島などの外地があり，それら外地における漁業情報は内地漁業とは区別されて当時の統計表に記載されている。資源解析という意味合いにおいては，これら外地における漁獲量をすべて含めた漁獲量を扱うことが好ましいことは明らかである。しかし，これら外地の情報は露領を除き，農林省が関東庁，朝鮮総督府，台湾総督府，南洋庁に照合して得たものであり，多くの場合，漁獲量は総数のみの数量，金額が記載され，魚種別内訳が不明であるため（漁獲量累年統計表，1960），本報では参照せず，内地の漁船および漁獲情報のみを扱った。本報で沖合もしくは遠洋と記したのは，それぞれの年代における統計表に，遠洋漁業の１（大正元年～大正６年），遠洋漁業の１（内地沖合）（大正７年～昭和４年），および内地沖合遠洋漁業（昭和５年～15年）と記載されたものであり，総トン数５トン以上の船舶およびその漁獲を意味する（漁獲量累年統計表，1960）。

なお，沖縄県の漁業情報（隻数や漁獲量）に関しては，戦時中の昭和19年から記載がなく，戦後も本県が占領下にあった期間（1972年に本土復帰）には本県の情報は統計表に含まれていないため，本報の漁船数や漁獲量に関して，これらの期間においては，沖縄県は含まれていない。

漁船数

戦前の漁船統計としては，大別してサイズ別漁船数（明治27年以降）と沖合（遠洋）漁業の漁業種類別漁船数（明治38年以降）の情報があり，どちらも動力船か無動力船かの仕分けがされているが，漁業種類別のサイズ別漁船数については記載されていない。漁業種類別になっていない漁船サイズ別統計は，資料としては利用が限定されるため，ここでは沖合･遠洋漁業に関する漁業種類別漁船数のみについて扱う。この漁業種類別漁船数は，明治38年（1905年）以降から編纂されてはいるものの，現在とほぼ同様の漁業の分類形式が用いられるようになったのは大正４年（1915年）であるため，本稿ではそれ以降の情報を用いた。

Fig. 1に沖合（遠洋）漁業の漁船数の変化を無動力（人力・帆走）および動力別に示した。大正４年（1915年）の段階で，無動力と動力の隻数がすでにほぼ等しく合計で2,300隻ほどであるが，その後動力船が急増，無動力船が徐々に減少し，昭和５年（1930年）には合計9,258隻のうちの8,660隻（93%）が動力船となっている。近代の沖合および遠洋漁業の発達において，漁船の動力化が大きな役割を果たしていることを示すものである。かつお釣漁業においてエンジン付の漁船が誕生したのは明治39年の静岡県水産試験場の“富上丸”

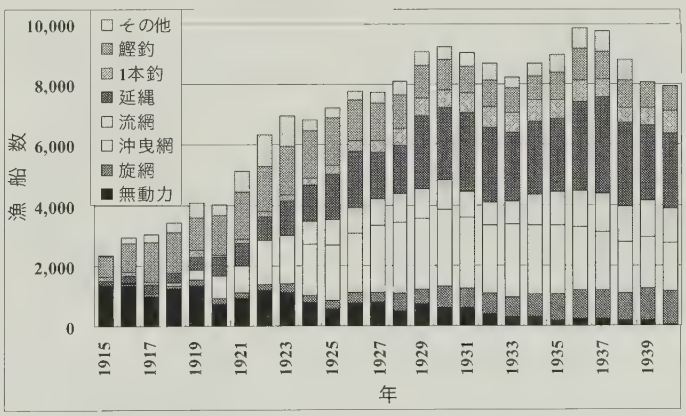

Fig. 1. 沖合漁業における無動力船および動力船（漁法別）の隻数の年変化
図中，鰹釣はいわゆる「かつお１本釣」を１本釣は「あじ・さば１本釣」を示すと思われる

Change in the number of non-motorized and motorized offshore fishing vessels. Motorized vessel was broken down into seven fisheries. Vessel categories from the bottom of the legend are non-motorized vessel, surrounding net, trawl net, drift net, longline, angling, pole and line, and others.

の建造にさかのぼる。大正12年（1923年）には，かつお釣動力船は約1,600隻に達するが，その後，昭和6年（1931年）の900隻にまで減少しほぼ安定する。一方，はえ縄漁業の場合も，明治40年頃から動力化が始まったものの，本格的に広まったのは大正に入ってからのようである。大正末期にはほぼ9割が動力船となり，その隻数は昭和4年（1929年）には2,000隻を超えている。まぐろ類の漁獲を主とするはえ縄漁業の場合，その漁場は一般にかつお釣漁場よりも沖合であり，いわば動力化によりはえ縄漁業が急速に発達したのであろう。また，かつお釣漁船が大正末期以降，むしろ減少しているのは，一部のかつお釣船がはえ縄漁業に転向したのではないかと推察されるが確証はない。

漁獲量（水揚量）

かつお・まぐろ漁業にかかわる漁獲物として，ここではかつお類，まぐろ類，かじき類，さめ類を扱うが，戦前の統計表では，各グループは残念ながら魚種別の統計にはなっていない（魚種別の統計は1951年から作成されている）。かつお類にはカツオ（*Katsuwonus pelamis*）の他に，少なくともヒラソウダ（*Auxis thazard*）とマルソウダ（*Auxis rochei*）が含まれており，さらにスマ（*Eutynnus affinis*）やハガツオ（*Sarda orientalis*）が含まれている可能性がある。かつお類に関しては，年次によってはそうだがつお類が別集計された年次もあるが，ここでは統一して，そうだがつお類を含んだ値を示す。また，当時の漁場が赤道以北の中西部太平洋にほぼ限定されることから考えて，まぐろ類にはクロマグロ（*Thunnus thynnus*，大西洋クロマグロは含まれていない），ビンナガ（*Thunnus alalunga*），メバチ（*Thunnus obesus*），キハダ

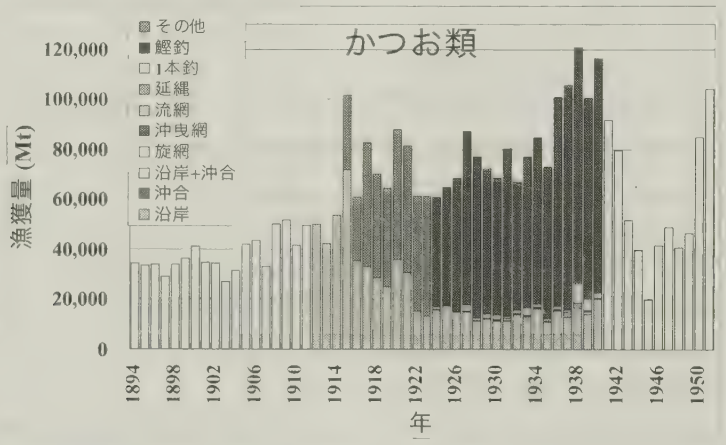

Fig. 2. かつお類の漁業種類別漁獲量の年変化1894～1951年
1924～1940年の沖合漁業についてのみ，漁法別に示した

Annual catch of skipjack tuna (including frigate and bullet tunas) from 1894 to 1951. Catch of offshore fishery from 1924 to 1940 was broken down into seven sorts of fisheries. Categories of the fishery from the bottom to the top are coastal, offshore, coastal+offshore, surrounding net, trawl net, drift net, longline, angling, pole and line, and other. The same fishery categorization and marks are used in Figs. 4, 6 and 8.

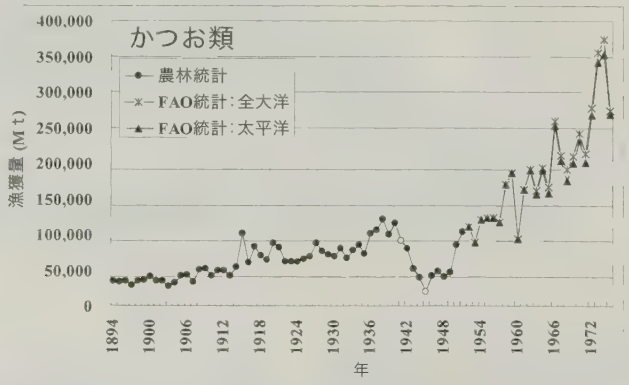

Fig. 3. 1894～1951年（農林統計）および1952～1975年（FAO 統計）における日本のかつお類の漁獲量変化
2つの白丸は開戦と終戦年を表す

Annual catch of skipjack tuna from 1984 to 1975. Two open circles indicate start and end year of World War II. FAO statistics was used from 1951-1975 for the catch of all oceans (star) and for that of all Pacific (triangle).

(*Thunnus albacares*) が，かじき類には，メカジキ (*Xiphias gladius*)，マカジキ (*Tetrapturus audax*)，クロカジキ (*Makaira mazara*)，シロカジキ (*Makaira indica*)，バショウカジキ (*Istiophorus platypterus*) およびフウライカジキ (*Tetrapturus angustirostris*) が含まれると推察される。さめ類に関しては，沖合・遠洋漁業での漁獲種としては，ヨシキリザメ (*Prionace glauca*)，クロトガリザメ (*Carcharhinus falciformis*)，ヨゴレ (*Carcharhinus longimanus*)，アオザメ (*Isurus oxyrinchus*)，ネズミザメ (*Lamna ditropis*)，おながざめ類などが大部分を占めると思われるが，沿岸漁業ではホシザメ (*Mustelus manazo*) やアブラツノザメ (*Squalus acanthias*) をはじめとする小型の沿岸種が多く含まれるものと考えられる。Fig. 2，Fig. 4，Fig. 6および Fig. 8にかつお類，まぐろ類，かじき類およびさめ類の漁獲量変化を1894年～1951年について示した。1894年～1911年および戦中戦後の1941年～1951年は漁業種類別の漁獲量は不明であるが，1912年～1923年は沿岸漁業と沖合漁業別に，1924年～1940年は沖合漁業をさらに漁業種類別に分割した漁獲量が記録されている。1894年～1911年に関しては漁業種類に関する情報はないが，大半は沿岸漁業による漁獲であろうと推察される。また，戦前のそれら魚種グループにおける漁獲量が，1952年以降急速に発達した遠洋漁業によりもたらされた漁獲量に比べて，どの程度の水準であったのかを把握するために，1952年以降の各グループの全大洋および太平洋における我が国の漁獲量の年変化を FAO 統計から参照し，それ以前の漁獲量とともにグラフで示した (Fig. 3, Fig. 5, Fig. 7, Fig. 9)。FAO 統計に関しては，以下

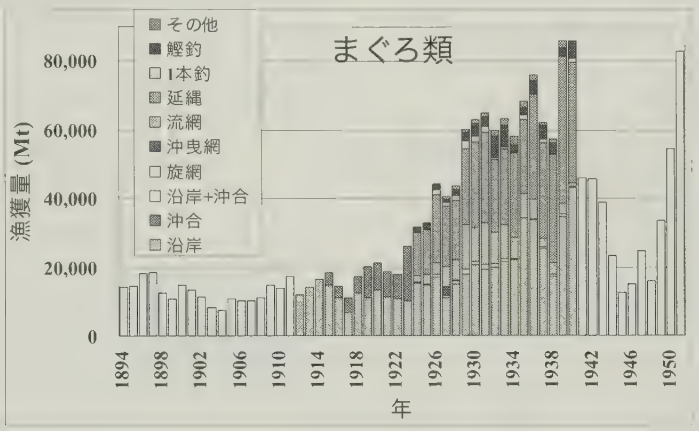

Fig. 4. まぐろ類の漁業種類別漁獲量の年変化1894～1951年
1924～1940年の沖合漁業についてのみ，漁法別に示した
Catch in weight of tunas from 1894 to 1951. Catch of offshore fishery from 1924 to 1940 was broken down into seven sorts of fisheries.

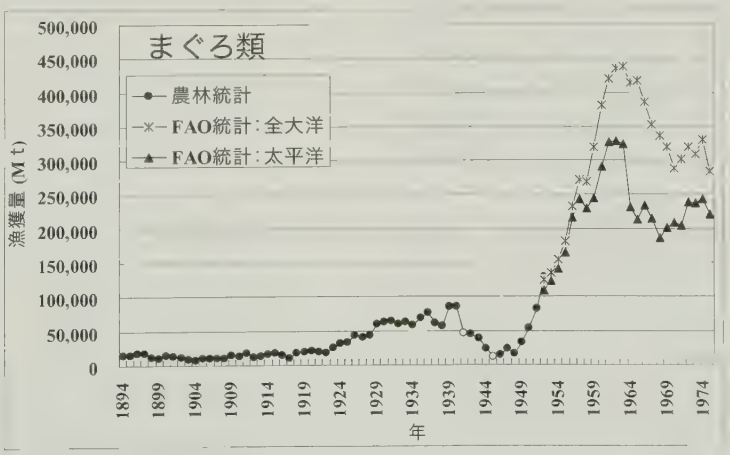

Fig. 5. 1894～1951年（農林統計）および1952～1975年（FAO 統計）における日本のまぐろ類の漁獲量変化
2つの白丸は開戦と終戦年を表す
Catch in weight of tunas from 1984 to 1975. Two open circles indicate starting and finishing year of World War II. FAO statistics was used from 1951-1975 for the catch of all oceans (star) and for that of all Pacific (triangle).

にホしたFAOのホームページからダウンロードした統計値を用いた。

http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp#Features

Ftp.fao.org/fi/stat/windows/fishplus/capdet.zip (1.6 Mb)

かつお類（Fig. 2）：1914年以前には，沿岸漁業を主体としたかつお類の漁獲量は3万～5万トンであったが，その後の沖合漁業の発達により，1915年～1935年には6万～8万トン，1936～1940年では10万トン以上へと増加している。沖合漁業において，本種のほぼすべてはかつお釣漁業によって漁獲されていることがわかる。終戦の1945年にはかつお類の漁獲は2万トンにまで落ち込むが，その後急激に増加し，1970年代前半には，太平洋における漁獲は30万トン前後に達した（Fig. 3）。

まぐろ類（Fig. 4）：1918年頃までは沿岸漁業による漁獲が主体であり，総漁獲量も2万トンを超えることはなかったが，沖合漁業の発達に伴い漁獲は急増し，1929年には6万トンを超えている。沖合漁業の中では，1920年代後半～1930年代前半に流し網が比較的多く（最大で約20%）の漁獲をあげているが，基本的には，はえ縄による漁獲が大部分を占めている。戦中戦後にかけて（Fig. 5），その漁獲量は1万トン台にまで落ち込むが，1960年代前半には，太平洋の漁獲だけでも30万トンに達するほど急激に増加している。

かじき類（Fig. 6）：かじき類の統計は他のかつお，まぐろおよびさめ類に比べて貧弱であり，統計表にはじめて登場するのも大正11年（1922年）である。遅くとも1920年代前半には沖合のはえ縄漁業により，かじき類の漁獲量もかなり増加していたものと思われるが，1940年までは沿岸漁業の漁獲量（漁業種類別には

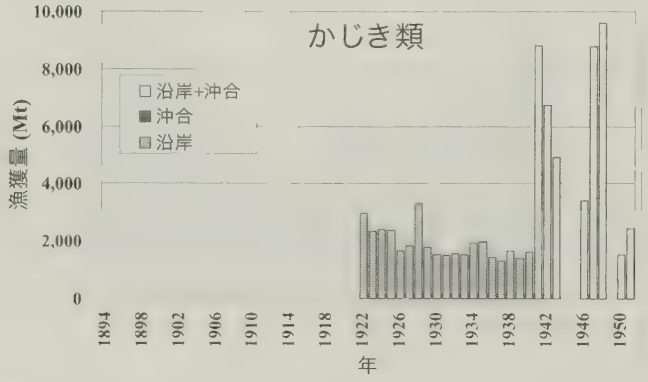

Fig. 6. かじき類の漁業種類別漁獲量の年変化　1922～1951年
1921年以前の漁獲量は記載されておらず，1922～1940年は沿岸漁業の漁獲のみが記載されている
Annual catch of billfishes from 1922 to 1951. The catch of billfishes was not recorded before 1921, and that of only coastal fishery was recorded from 1922 to 1940.

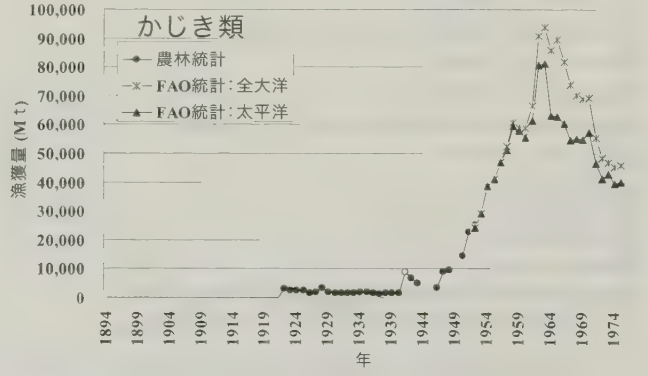

Fig. 7. 1922～1951年（農林統計，1944, 45, 49年はデータ無し）および1952～1975年（FAO統計）における日本のかじき類の漁獲量変化
2つの白丸は開戦と終戦年を表す
Catch in weight of billfishes from 1922 to 1975. Two open circles indicate starting and finishing year of World War II. FAO statistics was used from 1951-1975 for the catch of all oceans (star) and for that of all Pacific (triangle)

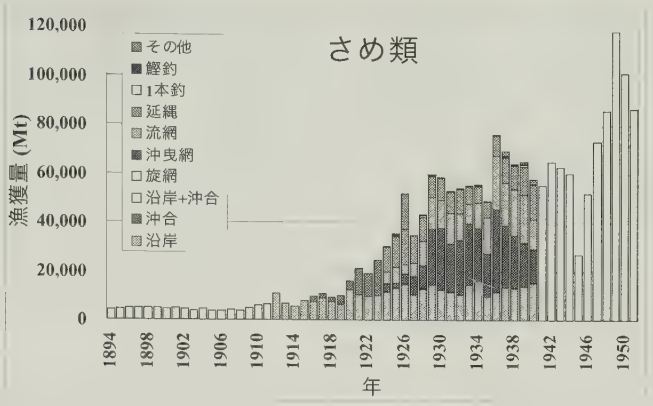

Fig. 8. さめ類の漁業種類別漁獲量の年変化 1894～1951年
1924～1940年の沖合漁業についてのみ，漁業種類別に示した
Annual catch of sharks from 1894 to 1951. Catch of offshore fishery from 1924 to 1940 was broken down into seven sorts of fisheries.

Fig. 9. 1894～1951年（農林統計）および1952～1975年（FAO 統計）における日本のさめ類の漁獲量変化
２つの白丸は開戦と終戦年を表す
Catch in weight of sharks from 1984 to 1975. Two open circles indicate starting and finishing year of World War II. FAO statistics was used from 1951-1975 for the catch of all oceans (star) and for that of all Pacific (triangle).

なっていない）しか掲載されておらず，1941年以降には沿岸漁業と沖合漁業の合計漁獲量（最大9,000トンほど）が示されているが，1944年，1945年および1949年の漁獲量は不明である。戦後，かじき類の漁獲量は急増し，1962年および1963年には太平洋だけでも８万トンに達するが，その後減少に転じ，1975年には約４万トンになっている（Fig. 7）。

さめ類（Fig. 8）：今でこそ，さめ類は練り製品の材料以外では人気の食用魚とはいい難いが，戦前，冷凍・冷蔵設備が十分ではなかった時代には食料用として，今よりも身近な魚であったと思われるし，鱶鰭（フカヒレ）は，当時から中国向けの重要な輸出品であった（樽本，1984）。そのためか，さめ類の漁獲統計はかじき類とは異なり，かつお類やまぐろ類と同じほどの詳細さで収集されていたものと思われる。その漁獲量は明治40年（1908年）頃までは4,000トン前後であったのが，その後の沖合漁業の発達により昭和４年（1929年）にはほぼ６万トンに達している。沖合漁業における漁業別漁獲量を見ると，さめ類は主にはえ縄，流し網および沖曳網で漁獲されており，昭和４年（1929年）からおよそ10年間は沖曳網での漁獲が最も多かったことがわかる。沖曳網は一種の底曳網であるから，主に小型のさめ類の漁獲をねらったものではないかと推察される。他の魚種と同様に，終戦の1945年には，その漁獲量は27,000トンにまで落ち込むが，戦後急速に増加し，1949年には約12万トンに達する（Fig. 9）。しかし，その後，さめ類の漁獲は減少を続け，1975年には昭和初期レベルの４万トンほどになる。この1950年頃からの漁獲の減少は，沖合漁業，特に北日本でのさめ類の漁獲を目的としたはえ縄漁が衰退したことと（樽本，1984），はえ縄が遠洋へと進出するに伴い，漁獲された外洋性のさめ類（主にヨシキリザメ）が，漁獲物として船内に取り込まれることが少なくなったことに起因すると推察される。太平洋以外の大洋（インド

洋と大西洋）にはえ縄漁業が進出した1950年代前半から，主漁獲対象であるまぐろ類に関しては全漁獲と太平洋内の漁獲量の差が広がっているのに対し，さめ類では両者にほとんど差が生じていないことからも，遠洋はえ縄漁業においてさめ類が船内に取り込まれなくなってきたことが裏付けられる。

2）水産試験場調査船による操業情報記載されている内容と問題点

先述のように，水産試験場調査船による竿釣りおよびはえ縄の各操業のデータ，すなわち操業日，位置，表面水温，努力量，餌，漁獲量などの情報が昭和８年から昭和17年にわたって海洋調査要報第52報から71報に収録されている。この海洋調査要報は，大正７年から昭和26年までの間，戦前は水産講習所，次いで水産試験場がそして戦後の第73報からは東海区水産研究所によって半年に１冊発行され，その名前のとおり，主に海洋観測調査の結果がまとめられた書物である。一般に，漁業管理委員会等で用いられる漁獲統計は，先述の農林統計に代表される水揚統計と漁獲成績報告書などから編纂される漁獲の時期，位置，漁獲量が特定される漁獲量・努力量データに分けられるが，この水産試験場調査船の漁獲試験情報は著者が知る限り最も古い，系統だった漁獲量・努力量データである。ただし，努力量や漁獲位置などの必須情報で未記入も少なくなく（Table1），各船の航海ごとに記録されている内容，特に漁獲物に関する記述の様式が必ずしも統一されていない。加えて，その航海で漁獲があった種類しか漁獲結果の表の冒頭に名前が記されていないため，特に主要対象魚種以外では漁獲されなかったのか，漁獲されても記録がとられなかったのかが不明瞭である。また，漁獲生物名に関しては，まぐろ，かじき，さめ，と種別になっていなかったり，具体的にクロマグロ，マカジキ，ヨシキリザメのように標準和名で書かれていたり，さらには両者が混在すらしている。そこで，なるべく記載に忠実に入力したファイルをまず作成し，次いで，操業位置，同航海の他の操業での記述，同年前後もしくは前後年の同船の記述内容を参照して，記入されていないはえ縄努力量（鉢数，鈎数）に関しては推定し，「まぐろ」のように一般名で書かれた漁獲生物名に関しては推定できる場合には魚種名（標準和名）に変更した。竿釣において努力量は針数で記されており，未記入も多いが，現時点で未記入部分の推定作業は行っていない。操業地点については，地名もしくは地名＋方向＋距離（例えば，潮岬 SE200浬）で書かれていることも多く，地名の位置が特定できる場合には緯度・経度に直したが，「○○根」などのように位置の特定が困難な場合，および位置に記載が無く，前後の日にも操業が無い場合にはデータとしての使用を断念した。最終的に利用し得た操業数ははえ縄で5,302操業（年間およそ16～25隻，400～1,000操業），竿釣で3,315操業（年間およそ７～18隻，200～700操業）であった（Table 2）。漁獲量は，はえ縄，竿釣ともに尾数で記載されており，漁獲重量および魚体サイズは不明である。記載によっては漁獲物の大きさについて大，中，小などと付記してあるものもあるが，その基準が不明であるため参照しなかった。

Table 1. 海洋調査要報に掲載されている水産試験場調査船のはえ縄操業記録における鉢数と鈎数の記入率
Perecentage of the longline operation in which the number of baskets (left) and hooks (ritght) used were recorded

年	鉢数記入%	鈎数記入%
1934	78.5	9.4
1935	89.1	44.7
1936	89.9	65.1
1937	92.8	68.5
1938	97.3	65.1
1939	92.7	71.9
1940	95.3	59.7
1941	90.0	75.2
1942	100.0	97.6

はえ縄操業

努力量および魚種別漁獲量の分布

はえ縄操業の操業数の分布を Fig. 10に示した。

1934年から1937年頃までは操業は主に日本近海，北緯20度以北の北西太平洋および南シナ海に分布しており，西部太平洋熱帯域では操業は行われていなかったが，1938年以降，西部太平洋の北緯20度から赤道付近に操業が広がり始め，1940年以降では東経155度以東の北西太平洋における操業は姿を消し，主漁場は北緯10度から赤道付近までの西部太平洋（ヤップ，パラオ周辺とミクロネシア周辺）に移行している。まぐろ類の漁獲尾数の分布を見ると（Fig. 11），1937年までは北西太平洋におけるビンナガが主漁獲対象魚種であったが，その後，西部太平洋熱帯域に漁場が移るにしたがって，キハダが主対象になっていったことが伺える。このことは，調査船操業の漁獲物における種組成にもよく現れており，1939年以降ビンナガの比率が減少し，代わってキハダの比率が増加している（Fig. 12）。

「かつお・まぐろ総覧」によれば，昭和13年（1938

Table 2. 海洋調査要報に掲載されている水産試験場調査船のはえ縄および竿釣操業における隻数と操業数（利用可能と認められるデータのみ）

The number of vessels and operations recorded for longline (left) and pole and line (right) fishing of prefectural research vessels.

	はえ縄		竿釣	
年	隻数	操業数	隻数	操業数
1934	16	339	13	341
1935	26	660	18	592
1936	24	783	17	647
1937	25	928	17	697
1938	23	637	11	246
1939	21	644	11	383
1940	19	573	6	214
1941	18	491	7	195
1942	7	247	0	0
合計		5,302		3,315

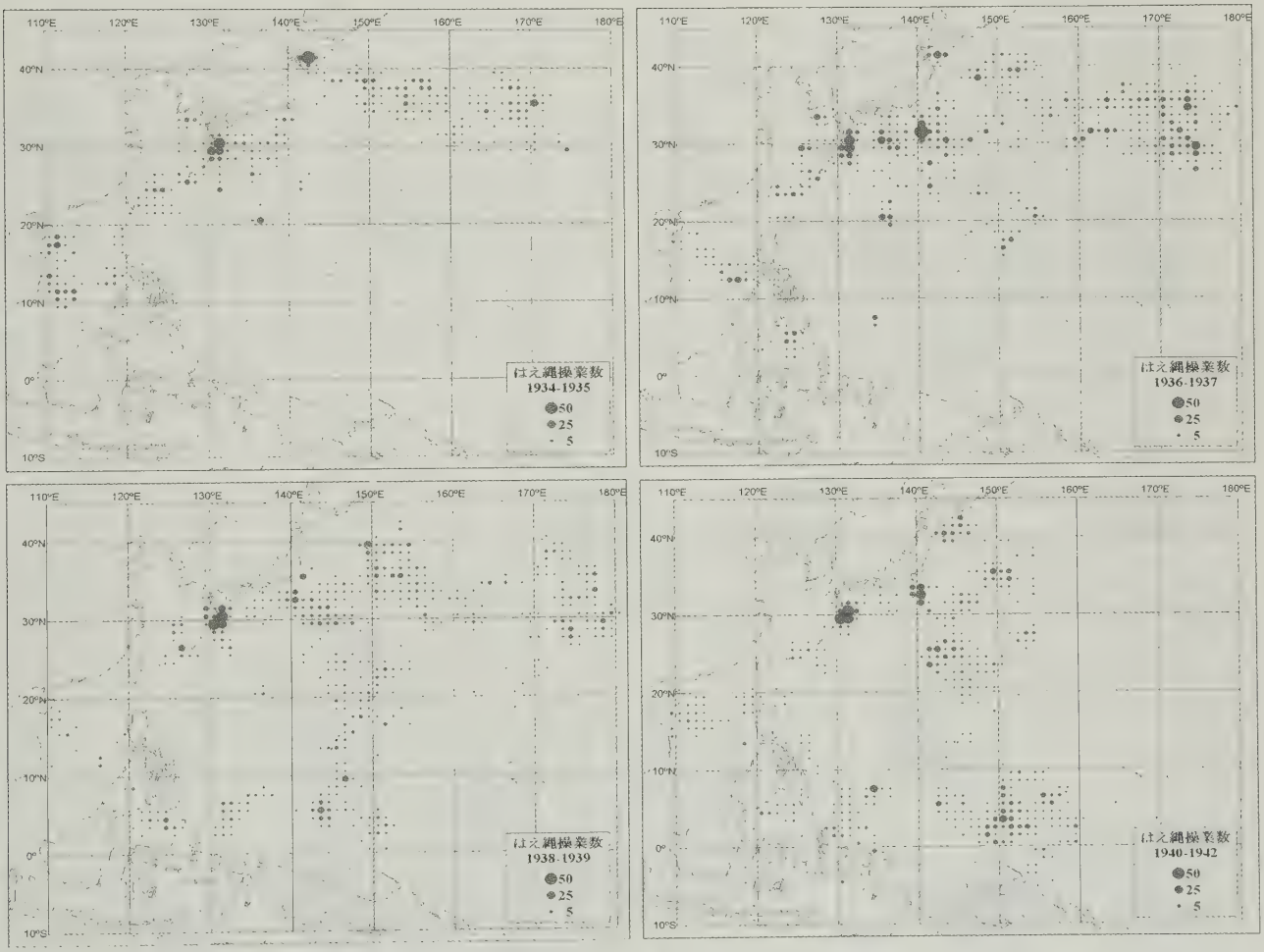

Fig. 10. 水産試験場調査船によるはえ縄操業の努力量分布（単位：操業回数）

Distribution of the number of longline operations conducted by prefectural research vessels

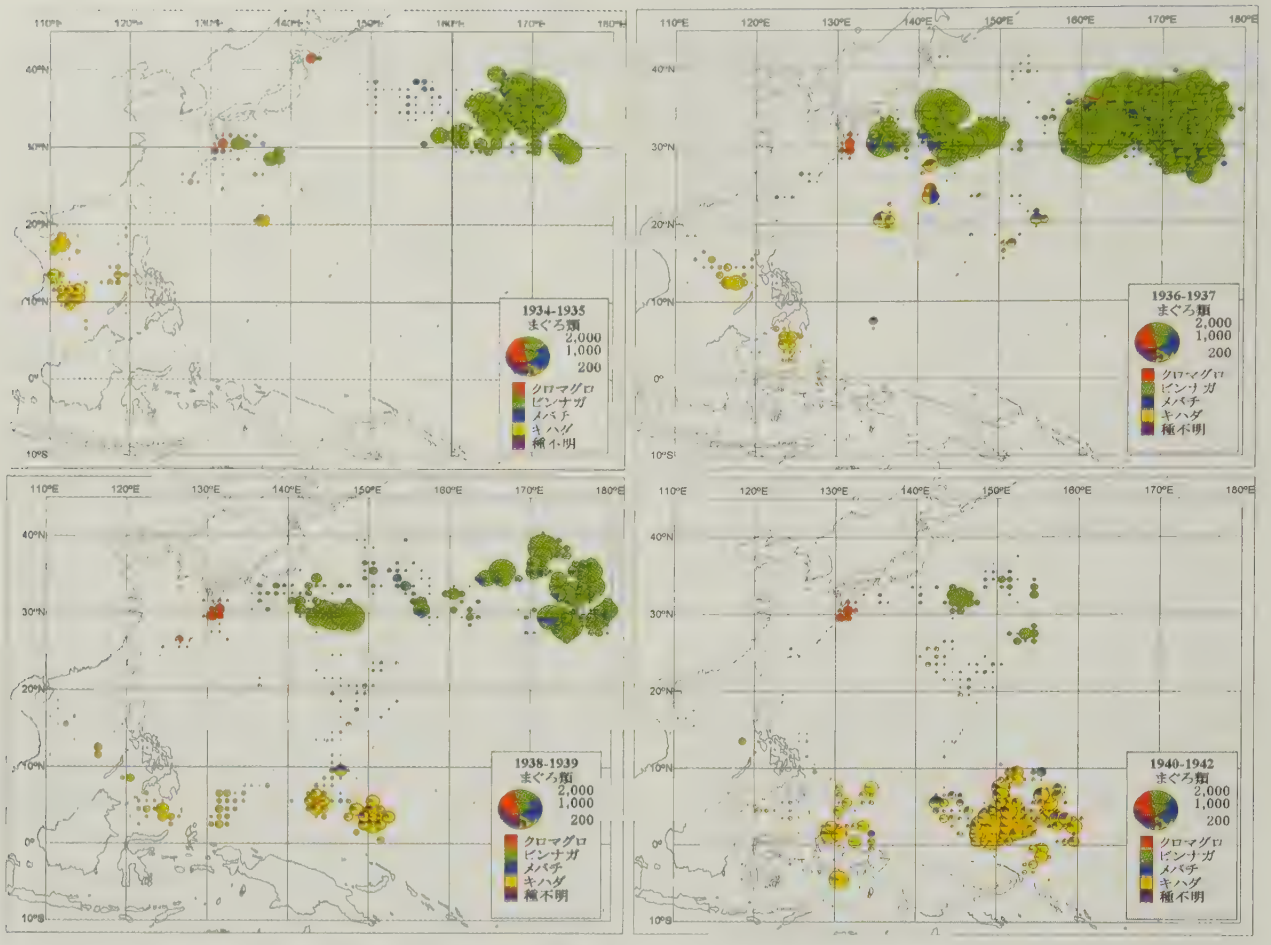

Fig. 11. はえ縄操業によるまぐろ類の魚種別漁獲分布
Distribution of catch in number of tunas by species (red: bluefin, green: albacore, blue: bigeye, yellow: yellowfin and purple: unknown) caught by longline operations of prefectural research vessels

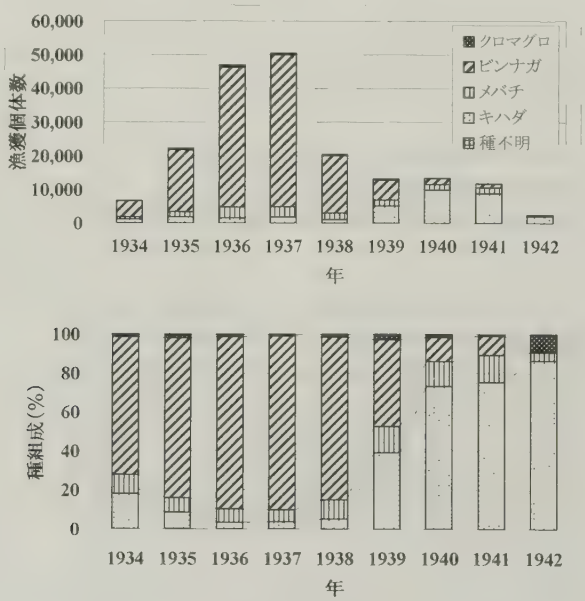

Fig. 12. はえ縄操業で漁獲されたまぐろ類における漁獲個体数（上図）と魚種比率（下図）の変化
Annual change of catch in number (upper figure) and species composition (lower figure) of tuna species (from the top of the legend, bluefin, albacore, bigeye, yellowfin and unknown) caught by the longline operation of prefectural research vessels.

年），１～２隻の一般はえ縄漁船が南方に進出して，非常に良い漁獲（おそらくはキハダ主体）を得，翌14年には76隻のはえ縄漁船が北緯20度以南に出漁したとされている。昭和13年頃まで好調だったビンナガ缶詰の対米輸出が両国の関係悪化のために困難となり，もはやビンナガ漁場に固執する必要がなくなったことがこの南方への漁場移動を促したようである。したがって，試験船の操業分布は比較的迅速に一般船の漁場の動きを追随しているものと思われる。他のまぐろ類の漁獲分布についても触れておくと，クロマグロの漁獲がすべての年を通して九州南方沿岸で認められ，メバチに関しては漁獲量および漁獲に占める割合は低いものの，ほぼ全操業海域で漁獲が認められる。

Fig. 13にかじき類の魚種別漁獲量分布を示した。漁獲量分布の特徴として，三陸沖の北緯35度～42度，東経145度～155度および伊豆諸島近海の北緯30度～35度，東経140度～145度付近でメカジキが多く漁獲され，1938年以降，北緯20度以南，東経140～160度の南方の海域でクロカジキが主に漁獲されていることが伺える。その種組成を見ても（Fig. 14），漁場の南下とともに1939年頃からクロカジキの比率が増加していることがわかる。マカジキに関しては，主に北緯20度～40度の間で漁獲され，北緯30度以北ではメカジキとともに，30度以南ではクロカジキとともに漁獲されている。

Fig. 15にさめ類の魚種別漁獲量分布を示した。東経140度～150度の北緯40度付近でネズミザメが，また北緯30度～42度のメカジキが多獲されている海域でヨシキリザメが多く漁獲されていることがわかる。しかしながら，図中に黄色で示したとおり，さめ類に関しては種名が記載されていないことが多いため，特に東経150度以東および北緯30度以南の種組成については不明確であり，同様に，種組成の年変化についても明瞭な傾向は認められない（Fig. 16）。

操業形態

はえ縄とは，幹縄と呼ばれる水平方向に張られたロ

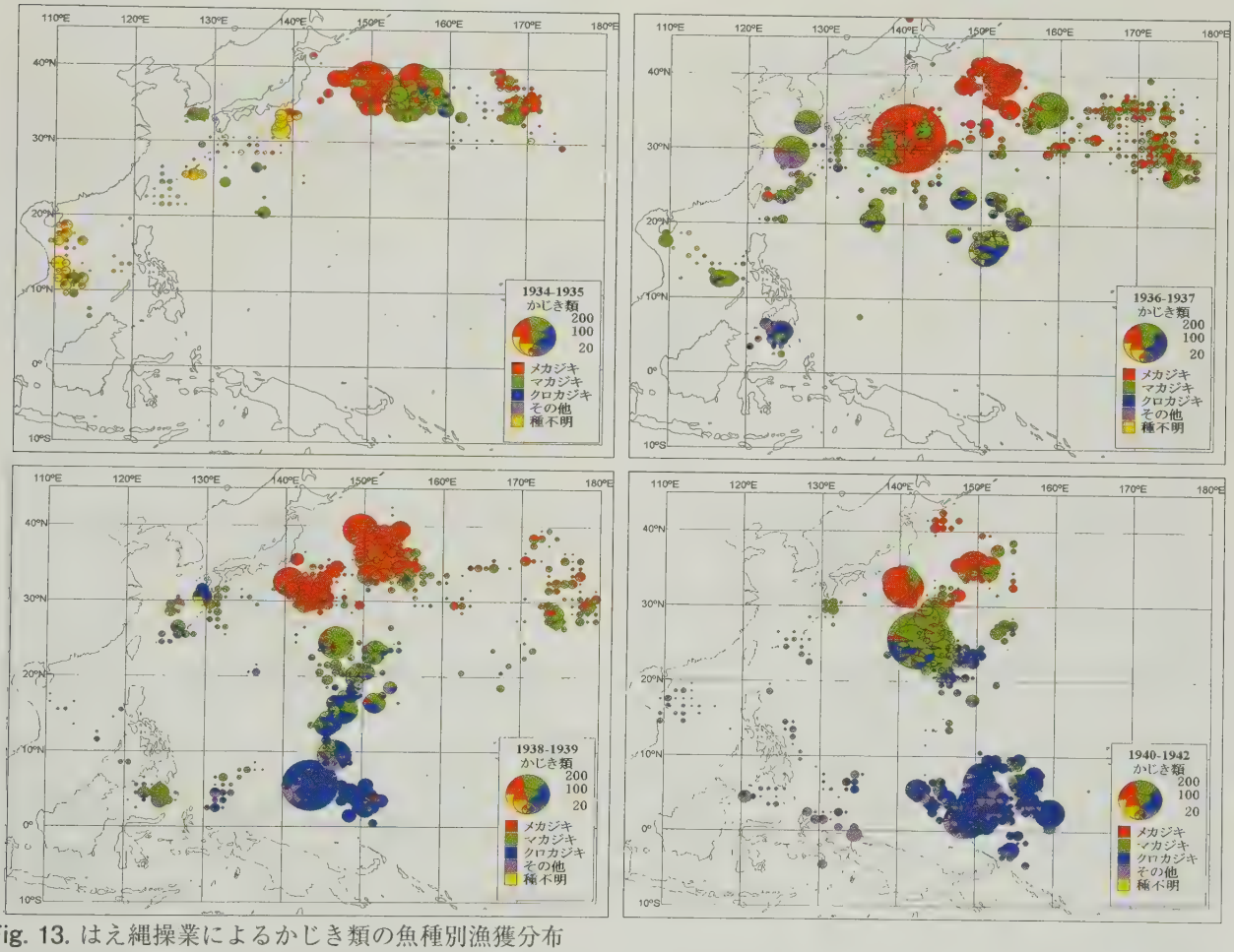

Fig. 13. はえ縄操業によるかじき類の魚種別漁獲分布
Distribution of catch in number of billfishes by species（red: swordfish, green: striped marlin, blue: blue marlin, pink: other marlin and yellow: unknown）caught by longline operations of prefectural research vessels

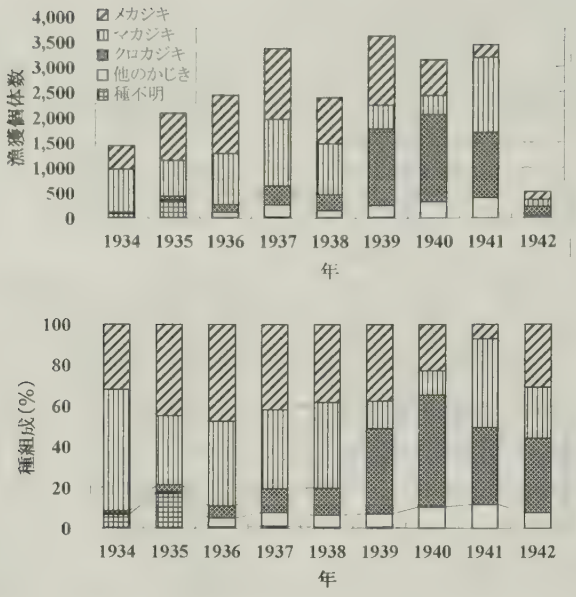

Fig. 14. はえ縄操業で漁獲されたかじき類における漁獲個体数（上図）と魚種比率（下図）の変化
Annual change of catch in number (upper figure) and species composition (lower figure) of billfish species (from the top of the legend, swordfish, striped marlin, blue marlin, other marlins and unknown) caught by the longline operation of prefectural research vessels.

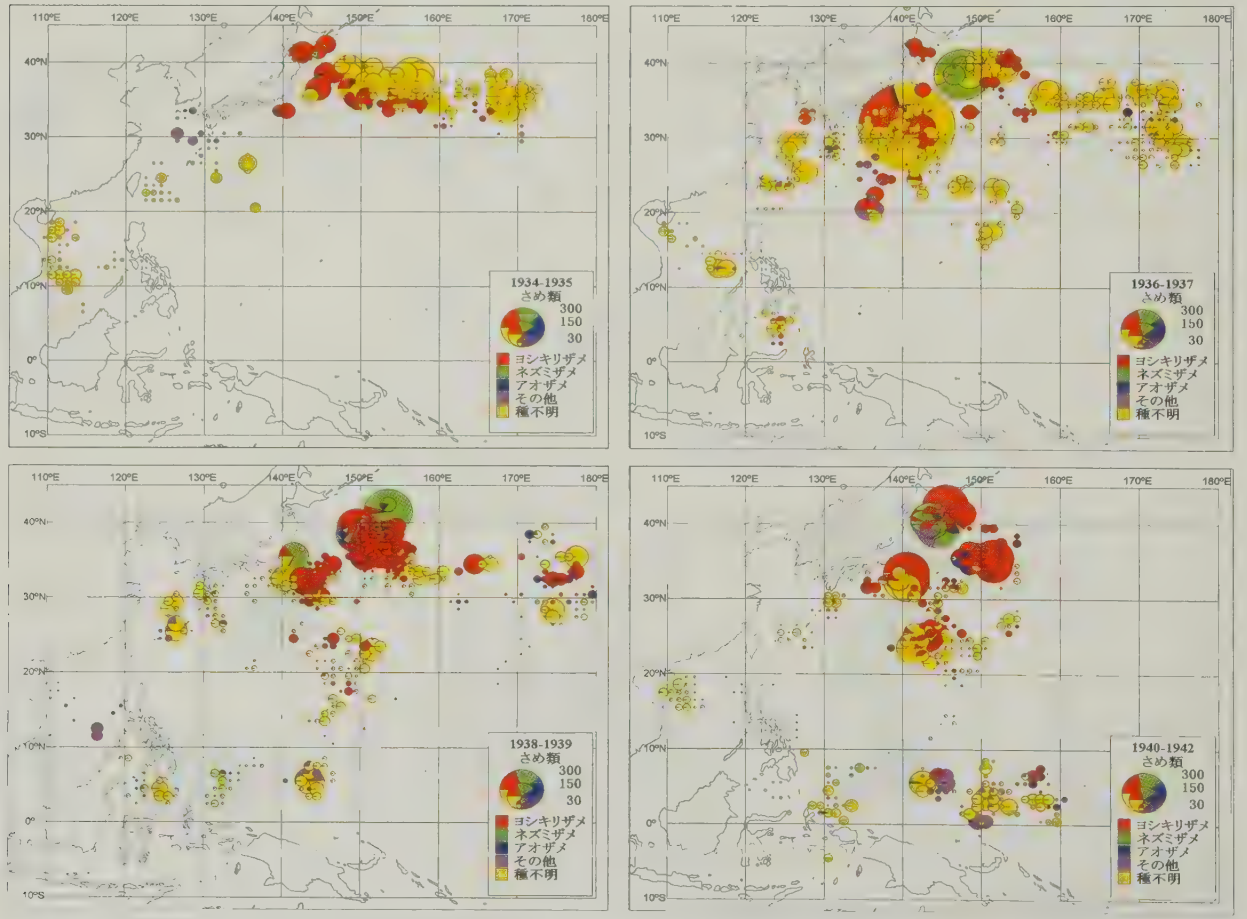

Fig. 15. はえ縄操業によるさめ類の魚種別漁獲分布
Distribution of catch in number of sharks by species (red: blue shark, green: salmon shark, blue: mako shark, pink: other sharks and yellow: unknown) caught by longline operations of prefectural research vessels

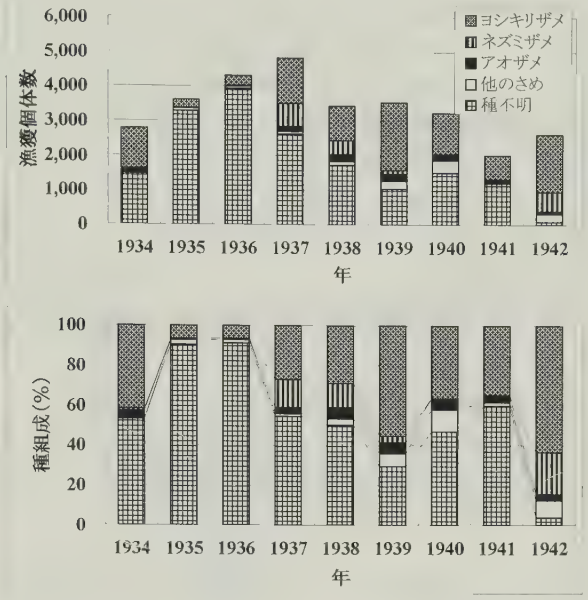

Fig. 16. はえ縄操業で漁獲されたさめ類における漁獲個体数（上図）と魚種比率（下図）の変化
Annual change of catch in number (upper figure) and species composition (lower figure) of shark species (from the top of the legend, blue shark, salmon shark, mako shark, other sharks and unknown) caught by the longline operation of prefectural research vessels.

ープを浮玉から伸びた受縄によって懸垂し，その幹縄から枝縄と呼ばれる先に釣鈎の着いた細い縄，もしくはテグスを多数吊り下げて魚を釣る漁法である。浮玉から次の浮玉までの間を通常，1鉢という単位で呼び，1鉢あたりの枝縄数すなわち鈎数によって「何本付け（1鉢あたり6鈎であれば6本付け）」，というように表される。1鉢あたりに用いられる枝縄数（鈎数）や枝縄，受縄の長さ，間隔などによって，漁具の設置水深が変わるため，それらの漁具構成は対象魚種や海域によって異なるし，さらに年代によっても変化が認められる。現在，近海・遠洋はえ縄操業で用いられている1鉢あたりの鈎数を対象魚種別に見ると，クロマグロとミナミマグロで8本前後，ビンナガでおよそ16本，メバチで15～20本，メカジキとさめ類で3～4本ほどである。この1鉢あたりの鈎数は1970年代半ばに大きな変化を示し，それ以前は6本以下が主流であったが，特に熱帯域のメバチ漁場において，遊泳深度のより深いメバチを効率よく漁獲するために10本付け以上のいわゆる“深縄”が用いられるようになった（Suzuki *et al.*, 1977）。

戦前のはえ縄操業の方法について具体的に総説された資料は無いので，記載された調査船の操業データから，当時の操業形態について紹介する。もちろん，調査船の操業がある程度，一般操業船の操業形態を反映しているであろう，という仮定が前提である。また，ここで紹介する操業情報については，未記入部分を補完した操業データは用いず，操業情報（鈎数，鉢数，操業時刻など）が記載されていたデータのみを使用した。

Fig. 17に試験船のはえ縄調査において，1操業あたりに使われた鈎数の分布を示す。1操業で使われた鈎数の範囲はおよそ100～2,000本であり，全体の約60%は600本以下である。現在，近海・遠洋はえ縄漁船で使われる鈎数が1,700～3,000本，メカジキ・さめ類漁場であれば3,000～4,000本であるのに比べれば極めて少ない。海洋調査要報には，調査船データが記載された全ての年ではないが，“委嘱船漁況調査報告”として，かつお釣およびはえ縄船の漁獲，水揚げ金額，操業方法などが航海単位でまとめられて紹介されている。それによれば，1操業で用いられる鈎数は，沿岸で300～1,500，沖合・遠洋で1,000～2,000と見られる。使用される鈎数は漁船の大きさにかなり依存するはずであるが，漁船サイズ別の情報は無い。調査船の場合，現在の調査船でも同様であるが，一般操業船とほぼ同レベルでの操業を行う船もあれば，調査が主で努力量自体がかなり少ない船もあり，その鈎数から当時の一般漁船の努力量を推し量るのは困難であろう。

1鉢あたりの釣鈎数を見ると，6本付けが圧倒的に多く全体の36%を占め，次いで2本付け16%，11本付け10%，4本および8本付けが約8%の順となっている（Fig. 18）。その分布を見ると，1～4本付け（大部分が2本付け）の多くが九州南方のクロマグロ漁を中心とした沿岸操業で多く用いられ，5～7本付け（多くが6本付け）が東経160度以西の本州沖合および南緯10度以南の南方漁場で，11本付け以上がビンナガの

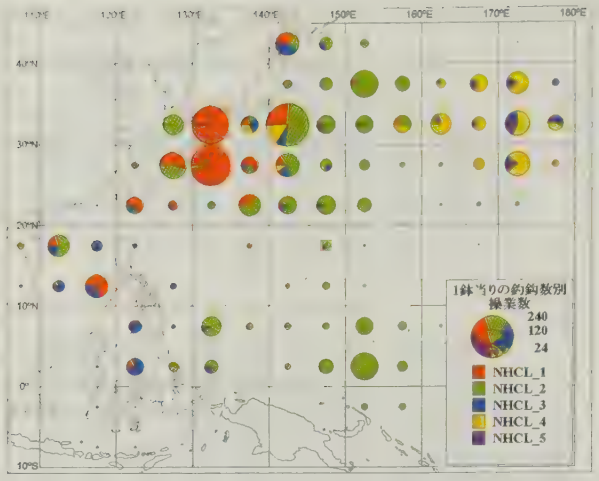

Fig. 17. 1鉢あたりの枝縄数（鈎数）別に示したはえ縄操業数の分布
NHCL1：1～4本付け，NHCL2：5～7本付け，NHCL3：8～10本付け，NHCL4：11～16本付け，NHCL5：17本付け以上.
Distribution of the number of longline operation by the number of hooks between floats. NHCL1: 1-4, NHCL2: 5-7, NHCL3: 8-10, NHCL4: 11-16 and NHCL5: 17- hooks between floats.

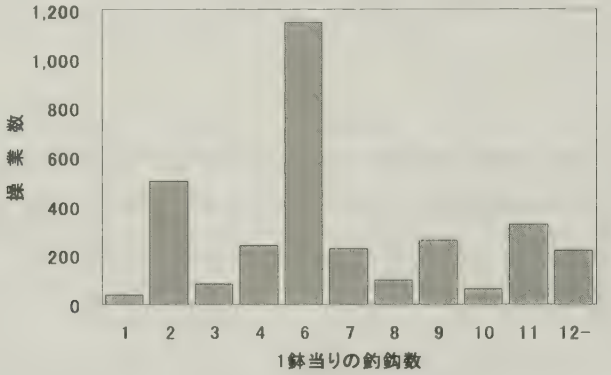

Fig. 18. 各1鉢あたりの釣鈎数で行われたはえ縄操業数
The number of longline operation by the number of hooks between float

主漁場である東経160度以東の北太平洋で用いられている。

今日のはえ縄操業は操業時間帯によって昼縄と夜縄に大別することができる。それぞれの典型的な操業時間帯をあげると，昼縄では午前2時～3時頃に投縄を開始し，午後2時～3時頃から10時間かけて揚縄を行い，夜縄では午後2時頃から投縄を行い，夜中の午前1時頃からやはり10時間ほどかけて揚縄を行う。いずれの操業においても，昼間もしくは夜間内だけで操業が完結するわけではなく，どちらの時間帯に長くに漁具の浸漬時間を設定するかの違いである。夜縄はメカジキおよびさめ類を狙った操業で用いられ，その短い枝縄と受け縄および少ない一鉢あたりの鈎数（3，4本付け）と合わせて，夜間に表層（40～70m）での漁獲を目的とし，昼縄は昼間により深い水深帯（70～300m）の漁獲を目的とすると言えよう。戦前の調査船における投縄開始時刻と揚縄開始時刻をプロットしてみると（Fig. 19），投縄開始が午前2時から10時，揚縄開始が午前10時から翌日の午前4時の範囲の操業と，投縄開始が午後3時から午前0時，揚縄開始が午前4時から午後2時の範囲の操業に大別されることがわかる。前者が昼縄操業に，後者が夜縄操業に相当すると思われるが，それぞれの時間帯の幅はかなり大きい。上記の投縄開始時間をもとに，昼縄，夜縄，その他の操業に分類し，その分布を見ると（Fig. 20），夜縄の分布は，メカジキが多く漁獲されていた海域（三陸沖と伊豆諸島近海，Fig. 13）にほぼ一致することがわかる。この海域はさめ類の好漁場でもあるため，メカジキもしくはさめ類のどちらを主対象とした操業かはわからないが，少なくとも当時からそれらの魚種は夜に多く漁獲されることが知られており，夜縄操業が一般的に行われていたことが示唆される。ただし，それら3種類（昼縄，夜縄，その他）に分けられた各操業で1鉢あたりの鈎数を観察すると（Fig. 21），夜縄

操業では7本付け以上の頻度は低いものの，他の操業と同じく，6本付けが最も多く用いられており，特にメカジキが，より浅い層で（夜間に）漁獲されるという認識はあまり無かったのでないかと推察される。ただし，漁具の構成，たとえば枝縄や受け縄の長さなどが夜縄と昼縄で異なっていた可能性もあるので断定はできない。

竿釣操業

はえ縄操業の場合とは異なり，竿釣では操業分布に年変化は認められず，いずれの年においても操業は日本周辺に限られている（Fig. 22）。Fig. 23にカツオの漁獲尾数の分布を示したが，本種が主漁獲対象であるため，その分布は操業分布とほぼ等しくなる。カツオおよびまぐろ類の漁獲数およびその種組成を見ても，漁獲の95%以上はカツオであり，まぐろ類の漁獲は取

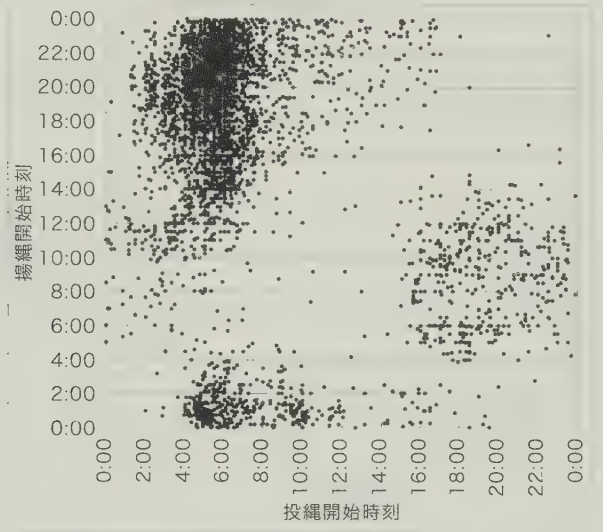

Fig. 19. 調査船のはえ縄操業における投縄開始および揚縄開始時刻の関係
Relationship of the start time of setting（X-axis）and hauling（Y-axis）longline gear

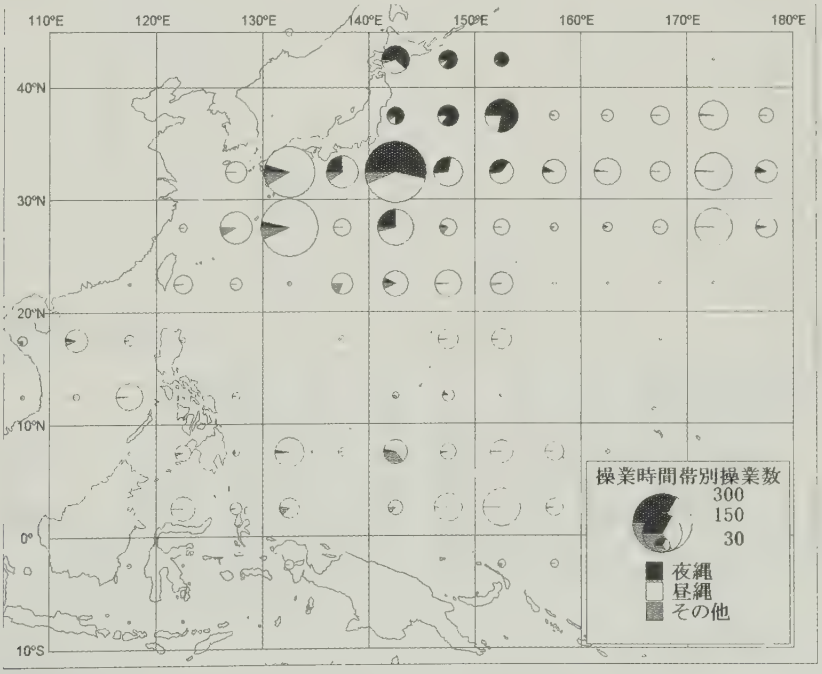

Fig. 20. 操業時間帯別操業数の分布
夜縄：投縄開始時刻15：00～01：00，昼縄：01：00～11：00，その他11：00～15：00
Distribution of night setting（time of start setting gear：15：00-01：00），day time setting（01：00-11：00）and other（11：00-15：00）longline operations.

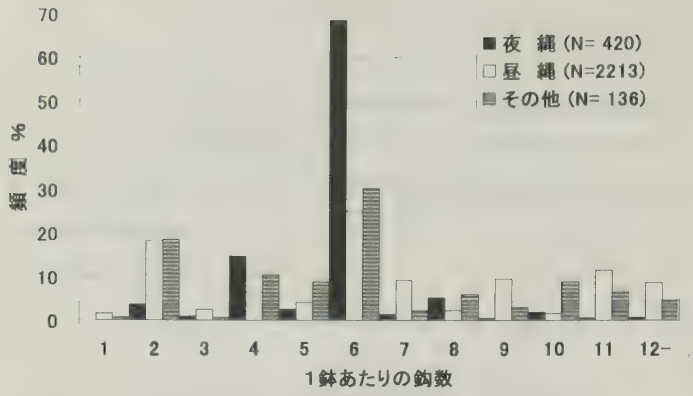

Fig. 21. 夜縄，昼縄およびその他の操業において用いられた1鉢あたりの釣鈎数の頻度%
Frequency (%) of the number of hooks between floats used for night setting, day time setting and other longline operations.

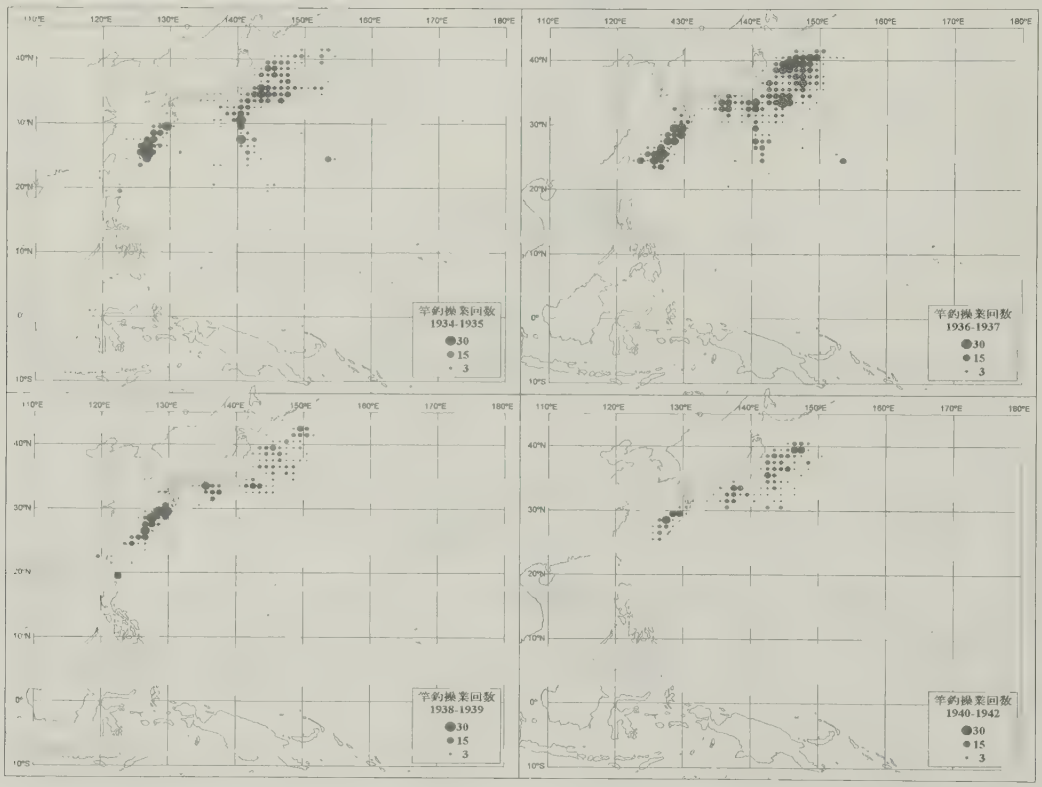

Fig. 22. 水産試験場調査船による竿釣操業の努力量分布（単位：操業回数）
Distribution of the number of pole and line operations conducted by prefectural research vessels

るに足らない（Fig. 24）。一般かつお釣漁業は昭和10年（1935年）には南洋諸島にまで漁場を拡大し（「かつお・まぐろ総覧」），パラオ，サイパン，トラック，ポナペ等を漁獲および加工の基地として操業を行い，昭和12年には3万4千トンの漁獲をあげていた（「南洋群島要覧」，「焼津水産史上巻」，「南興水産の歴史」）が，調査船の竿釣り操業はこれを追随していない。第一次世界大戦以後，日本領土となっていた南洋諸島における水産業の振興はむしろ国策であったと思われるが，かつお漁業の主力は依然として日本近海にあり，あえて調査船が南方まで調査に出かけ，鮮度の落ちたカツオを本土に持ち帰る必然性がなかったのではないかと推察される。かつお竿釣り漁業の場合，その操業は昼間に限られるし，比較するべき漁具の情報もないので，ここでは解析は行わない。

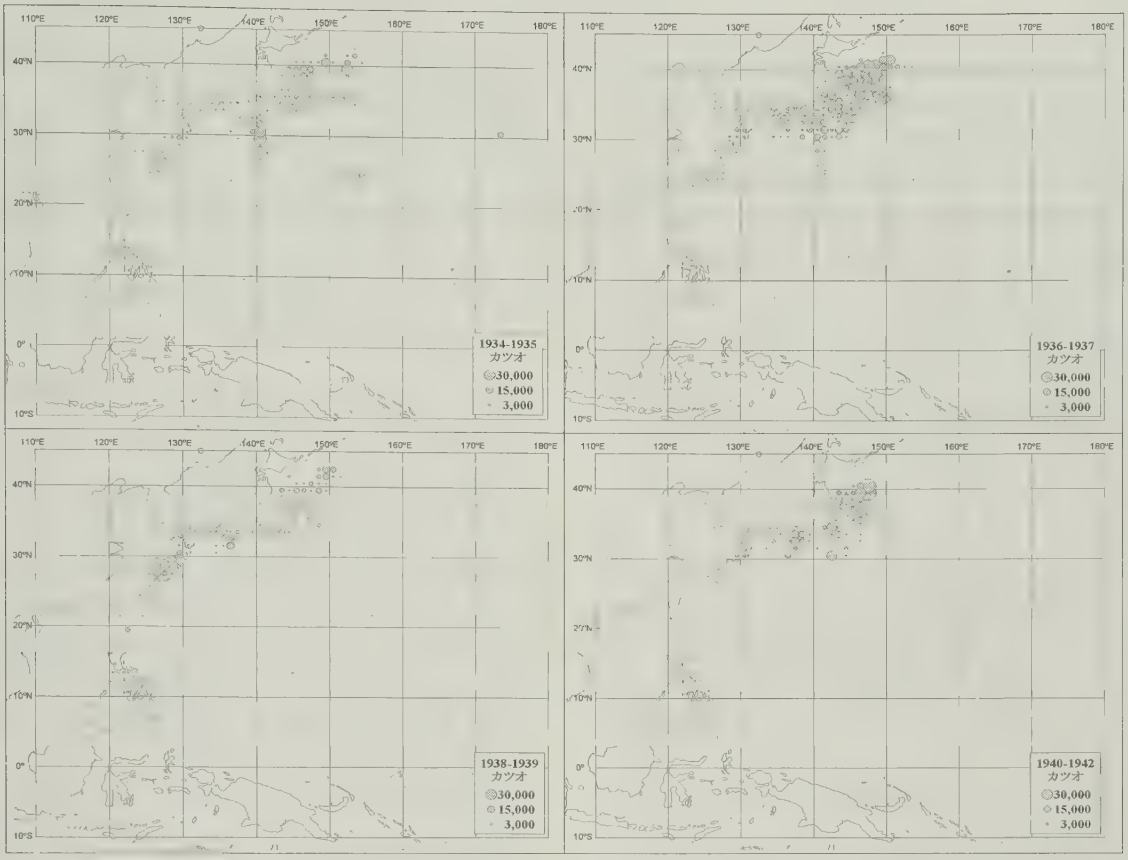

Fig. 23. 竿釣操業によるカツオ（Katsuwonus pelamis）の漁獲分布
Distribution of catch in number of skipjack tuna (Katsuwonus pelamis) caught by pole and line operations of prefectural research vessels

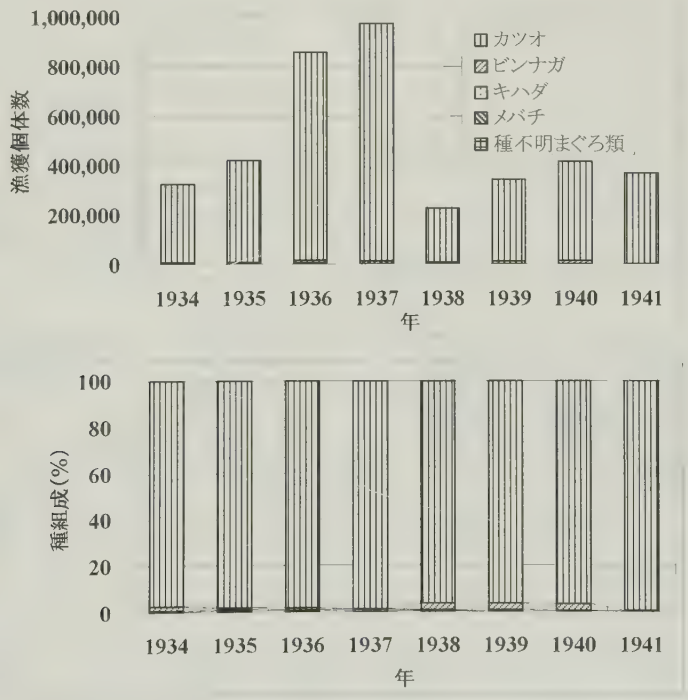

Fig. 24. 竿釣操業で漁獲されたかつお・まぐろ類における漁獲個体数（上図）と魚種比率（下図）の変化
Annual change of catch in number (upper) and species composition (lower figure) of skipjack and other tunas (from the top of the legend, skipjack, albacore, yellowfin, bigeye and unknown) caught by the pole and line operations of prefectural research vessels.

3．終戦直後のデータ収集状況

1945年8月15日ポツダム宣言受諾直後から日本の船舶はすべての活動が禁止され，およそ1ヵ月後には日本沿岸から12海里以内での航行および漁業活動が認められた。この制限区域の境界はマッカーサー・ライン（McArthur Line）と呼ばれ，Fig. 25に示したように段階的に拡張された。昭和27年（1952年），4月25日にすべての漁獲制限区域は開放され，日本の遠洋まぐろ漁業は全大洋にその漁場を急速に広げた。

終戦後すぐに，東北区水産研究所および南海区水産研究所の職員により，一般漁船（はえ縄と竿釣）の漁獲データの収集が開始された。今のように，漁船主から漁獲成績報告書が提出されるようになったのは1960年代に入ってからであり，終戦後10数年間は主要な水揚港において調査員が漁船の航海日誌もしくは「船頭ノート」から各操業における操業および漁獲の情報を書き写してデータを収集していた。1963年頃に，それまでに収集された漁獲情報の編纂が行われたが，その際，日本の遠洋漁業が始まったマッカーサー・ライン撤廃（1952年）以降のデータが優先的に編集された。

そのようなわけで，今でもなお，国際漁業委員会での資源解析には1952年以降の日本のはえ縄データが用いられている（竿釣に関しては若干遅れて，1968年以降のデータから編纂が始められた）。戦後から1951年以前のデータについては，今もなお手書きの記録用紙として保存されている。これらの半ば忘れ去られてきたデータについて，収集当時の状況を確認できるうちにデータ編纂作業を行いたい。

4．終わりに

戦前のかつお・まぐろ漁業の漁獲情報を探した結果，見つかったのは，上述した農林統計（および南洋群島要覧）における漁船および水揚統計と海洋調査要報に収録された水産試験場調査船による竿釣およびはえ縄操業データのみであった。農林統計の場合，漁獲は水揚データであるため，一般漁船の漁獲量は把握できるものの，資源評価に際して不可欠とも言える漁獲位置および努力量の情報が得られないし，少なくとも戦前の統計ではかつお，まぐろ，かじきおよびさめ類については魚種グループでまとめられており魚種別（メバチ，キハダなど）の情報は得られない。一方，

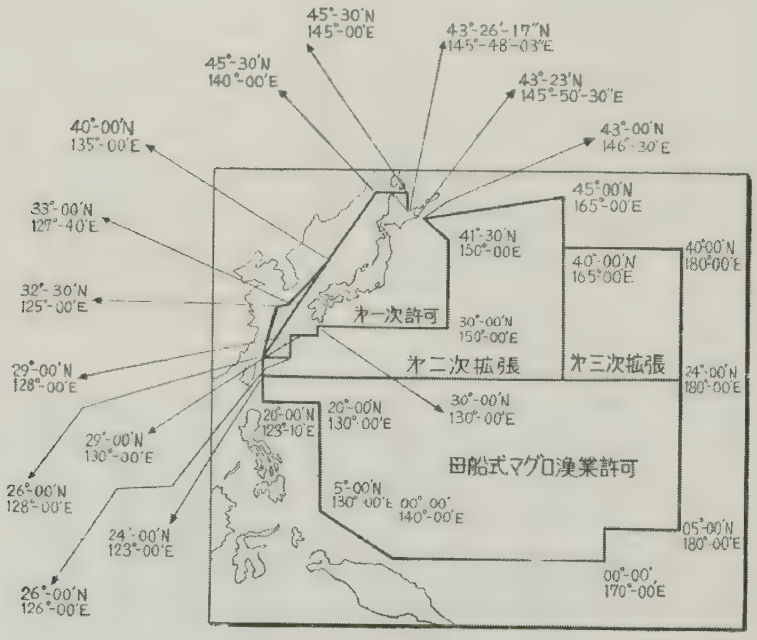

Fig. 25. マッカーサーラインの変遷 （「かつお・まぐろ総覧」より転載）
第1次拡張：昭和20年（1945）9月27日，第2次拡張：昭和21年（1946）6月22日，第3次拡張：昭和24年（1949）9月19日，母船式マグロ漁場の開放：昭和25年（1950）5月11日
Stepwise extension of the restricted Japanese fishing areas outlined by the MacArthur Line. 1st: 27th September 1945, 2nd: 22nd June 1946, 3rd: 19th September 1949, and the release of tropical fishing ground for the mother-ship longline fishery: 11th May 1950.

調査船データは年数も10年ほどと短いし，調査船の操業方法，努力量の地理的分布，および釣獲率がどれほど一般漁船のそれを反映しているのか，については疑問が残らざるを得ない。

そこで，魚種ごとの水揚データ，もしくは一般漁船の操業ごとの漁獲データが，どこかに保存されている可能性を探るべく，清水および焼津の魚市場，戦前から操業を行ってきている漁業会社およびすでに退職した漁業者等に問い合わせてみたが，残念ながら保存はされていなかった。たとえば，漁業資料館などに「浜帳」と呼ばれる水揚伝票のようなものが展示用に保管されているし，古い漁家に昔の「船頭ノート」などが眠っているかもしれないが，いずれも断片的な情報であろうし，資源解析での使用に耐えうるものとは考えがたい。

その他に系統だったデータが保存されている可能性としては，各水産試験場が収集した情報および海洋調査要報にも一部使用されている漁業無線の情報等が考えられるが，これらに関してはまだ直接的には確認していない。1953年に発行された南海区水産研究所報告第1号に，既往の資料からまぐろはえ縄漁業，特に戦前の漁場および漁況についてまとめられているが（中村，1953），そこで使われているデータも10数年間の調査船のデータのみであることからも，一般漁船の操業情報が，いかに収集されず，保管もされていなかったのかをうかがい知ることができる。様々な制約があるとはいっても，農林統計も調査船データも貴重な資料であることは間違いなく，調査船データにいたっては，各層水温や操業時間など，現在の漁獲成績報告書にも含まれていないような情報も含まれている。今後，これらのデータを戦後のデータとの比較，解析することによって，そのデータとしての利用可能性を探っていきたい。

謝　辞

本稿をまとめるにあたり，ご校閲いただいた遠洋水産研究所浮魚資源部および近海かつお・まぐろ資源部の職員の方々に深謝する。また，今回の仕事のきっかけとなった公庁船データの存在を教えていただいた中央水産研究所の渡邊朝生氏，戦前の漁獲データの保存状況について教えていただいた焼津漁業協同組合および株式会社清水魚（清水魚市場）の方々，戦前の漁業情報および終戦後のデータ収集について教えていただいた遠洋水産研究所元職員の藁科侑生氏，本間操氏，塩浜利夫氏，田中有氏，元はえ縄漁船船頭の川村春夫氏，統計資料の収集にご協力いただいた農林水産省図書資料室の方々に心から感謝する。

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Appendix Table 1. 本報で参照した統計資料の一覧
List of statistical bulletins cited in this paper

名称	出版年	統計年	編者
南洋群島要覧	1932～1943	昭和6年～昭和17年	南洋庁
農林省累年統計表	1955	明治1年～昭和28年	農林省農林経済局統計調査部
漁獲量累年統計表	1960	大正元年～昭和33年	農林省統計調査部
第1次農林省統計表	1926	大正13年	農林大臣官房統計課
～	～	～	
第18次農林省統計表	1942	昭和16年	農林大臣官房統計課
第1次農商省統計表	1943	昭和17年	農商大臣官房統計課
第2次農商省統計表	1944	昭和18年	農商大臣官房統計課
第11次農商務統計表	1896	明治27年	農商務大臣官房統計課
～	～	～	
第40次農商務統計表	1925	大正12年	農商務大臣官房統計課
海洋調査要報52報	1934	昭和8年1～6月	水産試験場
～	～	～	
海洋調査要報71報	1943	昭和17年7～12月	水産試験場

水研センター研報，第13号，35－77，平成16年
Bull. Fish. Res. Agen. No. 13, 35-77, 2004

博士号論文

Studies on molecular mechanisms underlying high pressure adaptation of α-actin from deep-sea fish

Takami MORITA *

Abstract Deep-sea fish distribute to depths of several thousand meters and at these abyssal depths encounter pressures that shallower-living fish cannot tolerate. Tolerance to abyssal pressures by deep-sea fish is likely to depend at least in part on adaptive modifications of proteins. However, structural modifications that allow proteins to function at high pressures have not been well elucidated. The objective of this study is to disclose the mechanisms underlying adaptation of deep-sea fish to high pressures. First, in order to select sample fish for this study, the author constructed the molecular phylogenetic trees for the deep-sea fish *Coryphaenoides* using the nucleotide sequences of the mitochondrial 12S rRNA and COI genes. The trees showed new arrangements of seven *Coryphaenoides* species with distinct groups, abyssal and non-abyssal species, that differed from previous taxonomic studies. Using the mutation rate of mitochondrial genes, the divergence time between abyssal and non-abyssal *Coryphaenoides* was calculated to be 3.2-7.6 million years ago. The present study suggests that hydraulic pressures play an important role in the speciation process in the marine environment. Second, the author cloned cDNAs encoding α-actin, which was used as a model protein to elucidate the mechanisms involved in protein adaptation to high pressures, from two abyssal *Coryphaenoides* species, *C. armatus* and *C. yaquinae*. Consequently, the author identified three amino acid substitutions, V54A or L67P, Q137K and A155S, that distinguished these abyssal α-actins from orthologs from non-abyssal *Coryphaenoides*. Finally, the author examined by several biochemical analyses which of the three substitutions makes possible for α-actin of the deep-sea fish adapt to high hydrostatic pressures. It was found that the substitutions of Q137K and A155S prevent the dissociation reaction of ATP and Ca^{2+} from being influenced by high pressures. In particular, the substitution of Q137K results in a much smaller change in the apparent volume for Ca^{2+} dissociation reaction. The substitution of V54A or L67P reduced the volume change associated with actin polymerization and has a role in maintaining the DNaseI activity of actin at high pressures. Taken together, these results indicate that a few amino acid substitutions in key functional positions can adaptively alter the pressure sensitivity of abyssal proteins.

Key Words: deep-sea fish, high pressure adaptation, actin, *Coryphaenoide*, molecular phylogenetic tree

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Introduction

Chapter 1. Molecular phylogenetic relationships of the deep-sea fish *Coryphaenoides* (Gadiformes: Macrouridae)

Chapter 2. Characterization and structure of

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注：東京大学審査学位論文（掲載に際し投稿要領に沿って一部修正した）

d-skeletal actin of the deep-sea fish
Section 1
Characterization of the deep-sea fish α-actin under high pressure
Section 2
Molecular cloning of α-actin from the deep-sea fish *Coryphaenoides*

Chapter 3. Identification of amino acid residues enabling α-actin from the deep-sea fish to adapt to high pressures

Chapter 4. General discussion

Acknowledgments
References

Introduction

The oceans constitute the largest habitat on Earth. Seawater covers 71% of its surface to an average depth of 3800m. Continental shelves in the region of 0 to 200m depth cover approximately 5 % of the Earth's surface, whereas slopes of 200 to 3000m depth, abyssal areas of 3000 of 6000m depth, and hadal areas of > 6000m depth do 13 %, 51 % and < 2 %, respectively (Fig. 1). The total volume of the oceans is $1.368 \times 10^9 km^3$, providing living space estimated to be 168 times that offered by terrestrial habitats (Cohen, 1994). The immense volume of seawater contributes 0.24% of the total mass of Earth and has a major influence on its climate.

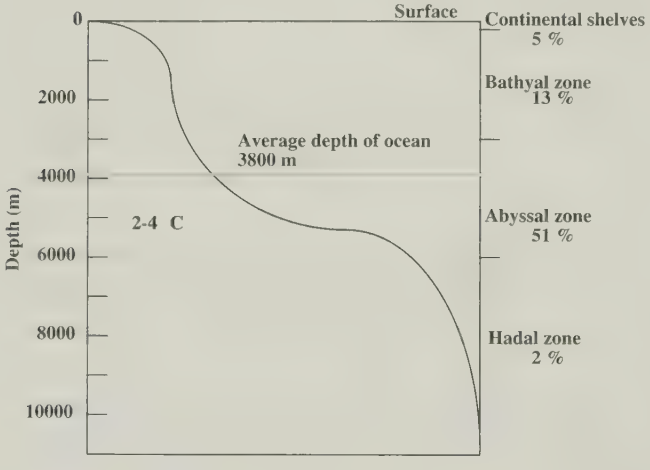

Fig. 1. Classification of the marine environments. The percentages refer to all Earth's surface area of the ocean floor included in each depth zone.

The deep-sea, defined variously as the ocean depths of 200 to 1000m, had been believed to be stagnant without life for a long time. But studies in the later 18th century revealed that it contains an abundance of life, often of bizarre forms (Mills, 1983). Though recent studies have clearly indicated that much more dynamic habitat exists than commonly believed (Gage and Tyler, 1991), the deep-sea had been considered to be a mysterious realm and organisms had been rarely explored.

The deep-sea is characterized by low temperature (1-4℃), extremely high hydrostatic pressures, non-photosynthetic light, and the relatively low influx of utilizable organic materials derived from the primary production in surface waters. These environmental factors endow organisms with some problems in colonizing this habitat. While some fish inhabit under the particular hydrostatic pressure, another experience various hydrostatic pressures during their life history. For example, certain fish spend early development stages in more productive area such as shallow waters, whereas vertical migration is prerequisite for reproduction of other fish species (Merret, 1978). However, most organisms inhabiting the deep-sea apparently have a characteristic bathymetric distribution throughout its geographic range. Therefore, the hydraulic pressure is considered to be especially important in the deep-sea life.

The hydrostatic pressure increases by 0.1 mega pascal (MPa) with the increase of every 10m depth in the ocean (Saunders and Fofonoff, 1976). Several different measurement units for pressure have been used in the literature. Recently the System International (SI) unit, Pa, has been authorized as a unit for pressure. The following conversion factors can be used: 1 atm = 1 bar = 14.7psi = 101325Pa. Hydrostatic pressures influence the function of proteins and biological membranes, especially in complex processes such as protein-protein interactions, enzyme-substrate binding, lipid-protein interactions, signal transduction, and biochemical correlates of organismal metabolism (Somero, 1990, 1992). Thus, hydrostatic pressures are considered

to play the critical role in vertical distribution of organisms and speciation in the deep sea, where homologous proteins from deep-sea fish tend to exhibit insensitivity to hydrostatic pressures (Siebenaller, 1991; Siebenaller and Murray, 1995; Childress and Thuesen, 1995; Gibbs, 1995).

Deep-sea fish distribute to abyssal depths of several thousand meters, the pressures of which shallower-living fish cannot tolerate. Tolerance to abyssal pressures by deep-sea fish is likely to depend at least in part on adaptive modifications of proteins. However, structural modifications that allow proteins to function at high pressures have not been well explored.

In the present study, the author made an attempt to give an answer to the classic, not old, and simple question: how do deep-sea fish adapt to high pressures of approximately 60 MPa ?

In Chapter 1, the author constructed the molecular phylogenetic trees of *Coryphaenoides* species by sequence analysis of two mitochondrial (mt) genes, *i.e.*, the 12S rRNA and cytochrome oxidase subunit I (COI) genes. Marine fish *Coryphaenoides* have a widespread bathymetric vertical distribution (Marshall, 1979) and have been extensively studied as model fish to elucidate the mechanisms involved in adaptation to the deep-sea (Smith, 1978; Siebenaller *et al.*, 1982; Stein and Pearcy, 1982). An excellent study system for efficiently investigating the environmental adaptation seems to compare the closely related species inhabiting different environments. A reliable molecular phylogenetic tree will create a way for selecting such sample species. The resent molecular phylogenetic trees based on mtDNA analysis indicated that abyssal *Coryphaenoides*, such as *C. armatus* and *C. yaquinae*, diverged 3.2-7.6 million years ago from non-abyssal species as the first event of *Coryphaenoides* speciation.

In Chapter 2, the author focused on the elucidation of the mechanism underlying high hydrostatic pressure adaptation of deep-sea fish. Many previous studies have identified proteins from deep-sea fish that function at high pressures (Swezey and Somero, 1985; Somero, 1990, 1992; Siebenaller, 1991; Siebenaller and Murray, 1995; Gibbs, 1997; Yancey *et al.*, 2001). One of these studies determined the volume change (δV) that is associated with polymerization of globular (G)-to filamentous (F)-state of α-actin from abyssal *C. armatus* and non-abyssal *C. acrolepis* and estimated the δV of actin from *C. armatus* was much smaller to be advantageous for a deep-sea habitat than those from *C. acrolepis* (Swezey and Somero, 1985). Actin is the major component of the microfilament system in all eukaryotic cells and plays a central role in maintaining cytoskeletal structure, cell motility, cell division, intracellular movements and contractile processes (Sheterline *et al.*, 1999; Pollard *et al.*, 1986). Thus, actin is one of the most conserved proteins in eukaryotic cells (Sheterline *et al.*, 1999). For example, the α-actin molecules from carp and rat share 99.4% homology at the amino acid sequence level (Collins and Elzinga, 1975; Watabe *et al.*, 1995). It is surprising that differences in the δV of this highly conserved protein have been found between two species of *Corypanenoides* that inhabit different niches. Therefore, the author expected that key amino acid substitution could allow the deep-sea fish to adapt to high hydrostatic pressures, and that such substitution would be easily found in comparison of actins from closely related species of *Corypanenoides* inhabiting different depths with a short divergence time. First, the author purified α-actin as a model protein for investigating high hydrostatic pressure adaptation from fast skeletal muscles of *C. yaquinae*, *C. armatus*, *C. acrolepis*, carp and chicken. Second, he cloned the α-actin cDNAs from *C. yaquinae*, *C. armatus*, *C. acrolepis* and *C. cinereus*, and determined their primary structures. He consequently identified three amino acid substitutions between non-abyssal and abyssal *Coryphaenoides*.

In Chapter 3, based on the structures of the actin molecules determined in Chapter 2 along with those previously reported, the author examined which of the three substitutions enables α-actin of the deep-sea fish to adapt to high hydrostatic pressures by using biochemical analyses, such as Quin 2, nucleotide exchange, and DNaseI inhibition assays. The results obtained indicate that the three amino acid substitutions provided deep-sea fish α

-actin with respective functions to cope with high pressures.

Chapter 4 is devoted to general discussion based on the results of this study and other related ones.

The present findings could contribute to better understanding of the formation of bathymetric distribution for marine organisms and the evolution of deep-sea organisms.

Chapter 1. Molecular phylogenetic relationships of the deep-sea fish *Coryphaenoides* (Gadiformes: Macrouridae)

In order to study efficiently the adaptation of proteins to high hydrostatic pressures, it is useful to compare closely related species inhabiting different depths. The divergence time among the closely related species among orthologs are relatively short. Therefore, it is predicted that a few amino acid variations among orthologs would allow the proteins to adapt to high hydrostatic pressures. An excellent study system for such purposes is provided by marine fish *Coryphaenoides*.

Marine fish *Coryphaenoides* are diverse and have a widespread bathymetric vertical distribution from 200 to 6400m in depth in the ocean. These are abundant in deep sea area of the Pacific ocean, and have been studied as model fish to elucidate their adaptation to the deep sea (Smith, 1978; Siebenaller *et al.*, 1982; Stein and Pearcy, 1982). Most *Coryphaenoides* species inhabit a restricted vertical range on continental slopes of 200 to 2000m deep in the ocean, whereas a few species are regarded as abyssal (Marshall, 1979). The present taxonomic arrangement of *Coryphaenoides* defining the subgenera has been based on only a few available taxonomic characters in morphological aspects (Iwamoto and Sazonov, 1988). The largest subgenus is *Coryphaenoides* (46 species), followed by *Chalinura* (8 species), *Nematonurus* (5 species), and *Lionurus* (2 species). The subgenus *Nematonurus* includes abyssal and non-abyssal species. *C. armatus* and *C. yaquinae* in the subgenus *Nematonurus* are known as abyssal species adapting to extremely high hydrostatic pressure among the genus *Coryphaenoides*, and their distribution ranges from 2700 m to 6400m deep in the ocean (Iwamoto and Stein, 1974). Biochemical phylogenetic approaches by peptide mapping of A_4-lactate dehydrogenase (A_4-LDH; 1.1.1.27) isoenzyme to study the evolutional adaptation of *Coryphaenoides* suggest that diversification of this genus began on the upper slope and then spread to the abyssal ocean (Wilson *et al.*, 1991; Wilson, 1994) (Fig. 2). Thus, phylogenetic relationships within the *Coryphaenoides* species are still unclear and have been disputed. The selection of candidate organisms considering phylogenetic relationships is important in comparative biochemical and molecular study (Garland and Adolph, 1994; Garland and Carter,

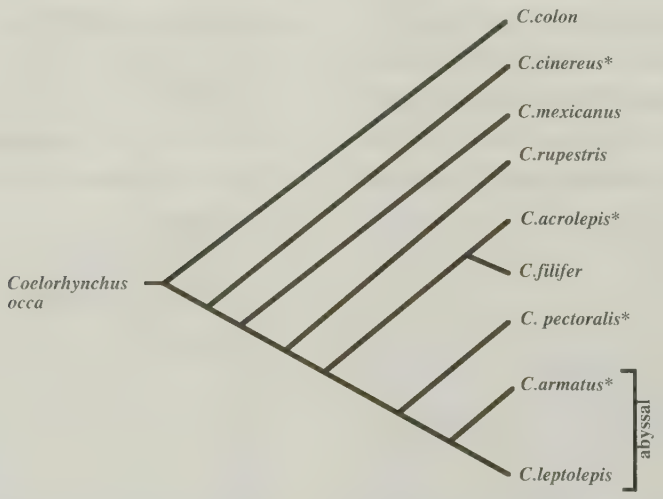

Fig. 2. Phylogenetic tree constructed by least-squares (Fitch-Margoliash) analysis based on peptide mapping of A_4-LDH homologs from *Coryphaenoides* species (Wilson *et al.*, 1991). *Species used in this study.

Table 1. Species used in this study

Family / Species	Collection site and depth (m)		
Macrouridae			
(Ingroup taxa)			
Coryphaenoides nasutus	38° 01.76′ N	142° 12.73′ E	810m
C. cinereus	38° 02.13′ N	142° 22.05′ E	997m
C. acrolepis	42° 04.00′ N	144° 42.80′ E	1960m
C. pectoralis	37° 21.35′ N	142° 18.42′ E	1001m
C. longifilis	37° 21.35′ N	142° 18.42′ E	1001m
C. armatus	44° 00.70′ N	145° 22.20′ E	3940m
C. yaquinae	39° 58.10′ N	154° 59.50′ E	5600m
(Outgroup taxa)			
Abyssicola macrochir	37° 58.81′ N	141° 48.97′ E	250m
Caelorinchus gilberti	37° 26.28′ N	142° 00.81′ E	603m

1994). A molecular phylogenetic tree based on the sequence analysis of mtDNA seems more suitable for such purposes (Meyer *et al*, 1990; Normark, 1991; Meyer, 1992; Fabrizio *et al*, 1996; Miya and Nishida, 1996; Ritchie *et al*., 1996; Miya *et al*., 2001).

In this chapter, the author constructed a reliable molecular phylogenetic tree of the genus *Coryphaenoides* by sequence analysis of two mitochondrial genes, *i.e*., the 12S rRNA and COI genes. The molecular phylogenetic tree will provide the guideline for selecting closely related species to investigate the adaptation of proteins from deep-sea fish to high hydrostatic pressures.

Materials and Methods

Materials and DNA extraction

Table 1 summarizes data of species used in this study. *C. armatus* and *C. yaquinae* were collected using large deep-sea pots with long lines by the research vessel (R/V) Soyo-maru of the National Research Institute of Fisheries Science. The other species were collected using trawl nets by the R/V Wakataka-maru of the Tohoku National Fisheries Research Institute. Total DNA was extracted from fast skeletal muscles tissues of frozen specimens by standard protocols using proteinase K digestion and phenol extraction (Kocher *et al*., 1989).

DNA amplification and DNA sequencing

Polymerase chain reaction (PCR) amplifications were performed in 100 μl of 10mM Tris-HCl buffer (pH 8.3) containing 1.5mM $MgCl_2$, 1 mM each of dNTP, 1 mM each of primers, 50-1000ng of template DNA, and 2.5units of Taq polymerase (Takara). The 12S rRNA gene was amplified with a primer set of L1091 and H1478 (Kocher *et al*, 1989). The other set of AAGCTATTATGATGGGCCCT (L640) and GTTCGAGTGAAGTACCATCA (H1110) were newly designed and used in this study. L and H refer to the sequences of light and heavy strands, respectively, and the numbers of the primers indicated the 3' end positions according to the numbering system for human mtDNA (Anderson *et al*., 1981). The COI gene was amplified with primers L6586 and H7086 (Palumbi and Wilson, 1990). PCR was performed at an initial denaturation step at 93 ℃ for 2 min, followed by 30cycles at 93℃ for 40sec, 55℃ for 1min, and 72℃ for 1.5min. The amplified fragments were purified on 1 % low-melting agarose gel. Each purified fragment was determined for its nucleotide sequences by direct sequencing of both sense and antisense strands with the PCR primers used for the DNA amplification as sequence primer. PCR was carried out using a Dye terminator cycle sequencing kit (Applied Biosystems) for 26cycles (at 96℃ for 30sec, 50℃ for 15sec, and 60℃ for 4 min). The cycle sequence products were precipitated with ethanol, resuspended in 4 ml of 50% formamide containing 0.1 M EDTA, and loaded onto an ABI 373A automated DNA sequencer (Applied Biosystems).

All sequences are available from the

DDBJ/EMBL/GenBank databases under accession numbers AB018224-AB018232 for the 12S rRNA gene and AB018233-AB018241 for the COI gene.

Phylogenetic analysis

The nucleotide sequences were aligned with the CLUSTAL W multiple alignment program (Thompson *et al.*, 1994) and refined manually. Maximum parsimony (MP) analyses were conducted with the computer program PAUP, test version 4d64 (Swofford, 1998). NEIGHBOR and DNAML in PHYLIP version 3.5 program package (Felsenstein, 1995) was used for neighbor-joining (NJ) (Saitou and Nei, 1987) and for maximum likelihood (ML) (Felsenstein, 1981). Distance matrices for the NJ analysis were estimated by the DNADIST program in the PHYLIP program package with the Kimura's two-parameter option (Kimura, 1980). Transition/transversion (TS/TV) rate was 4.0, according to the observed ratio within the ingroup and between the ingroup and outgroup (Fig. 3). MP was performed by an extensive search of the most parsimonious tree (s). Gaps were treated as missing data in all analyses. Bootstrap analyses (Felsenstein, 1985) were performed to examine the confidence of nodes within the resultant topology obtained by MP, NJ and ML analyses in 1000 times replicates. Abyssicola macrochir and *Caelorinchus gilberti* were used as outgroup species in the MP, NJ and ML analyses.

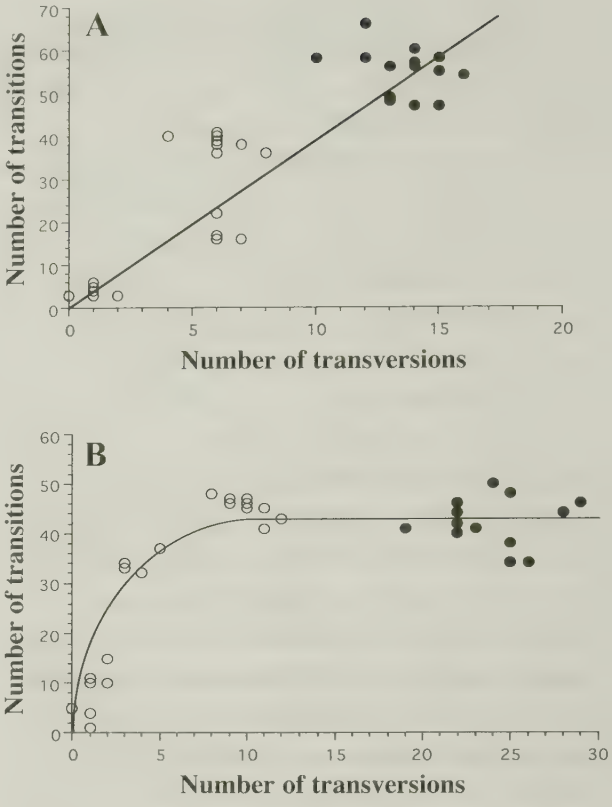

Fig. 3. Levels of saturation for base substitutions in the 12S rRNA and COI genes among the ingroup *Coryphaenoides* species and between *Coryphaenoides* species and outgroup species. (A) 12S rRNA and (B) COI (third codon position) genes. Open symbols indicate pairwise comparisons of transition vs. transversion within the ingroup, and closed symbols indicate those between ingroup and outgroup species.

Results

The nucleotide sequence of the 12S rRNA and COI genes corresponding to positions of 68 to 892 and of 6167 to 6611 of the Atlantic cod gene, respectively (Steinar and Ingrid, 1996) were determined for seven *Coryphaenoides* species (Fig. 4, 5).

The alignment among the determined sequences of the *Coryphaenoides* 12S rRNA gene was 829 bp in length, including 434 bp (52%) of stem sites and 395 bp (48%) of loop sites identified by comparisons with the secondary structure of the bovine 12S rRNA gene (Gutell *et al.*, 1985). The base composition among seven *Coryphaenoides* species were A = 22.3%, T = 22.4%, C = 26.7%, and G = 28.7% in the stem site and A = 39.9%, T = 22.4%, C = 22.7%, and G = 14.9% in the loop site. The stems of the fish genes showed a rough base frequencies, while loops had an excess of A and a low frequency of G, as noted in mammals (Springer *et al.*, 1995). Several insertions were observed in the loops, but only one insertion in the stems. Pairwise sequence divergence estimates ranged from 0.0036 to 0.0571 (mean = 0.0331) between each two pairs of species within the genus *Coryphaenoides* and from 0.0739 to 0.0947 (mean = 0.0885) in comparison between *Coryphaenoides* and outgroup species (Table 2). TS/TV ratio of the 12S rRNA gene among *Coryphaenoides* species and outgroup species ranged from 1.5 to 10.0 except for *C. pectoralis* and *C. acrolepis* having TV = 0. Since the TS/TV ratio of the 12S rRNA gene within the ingroup was constant,

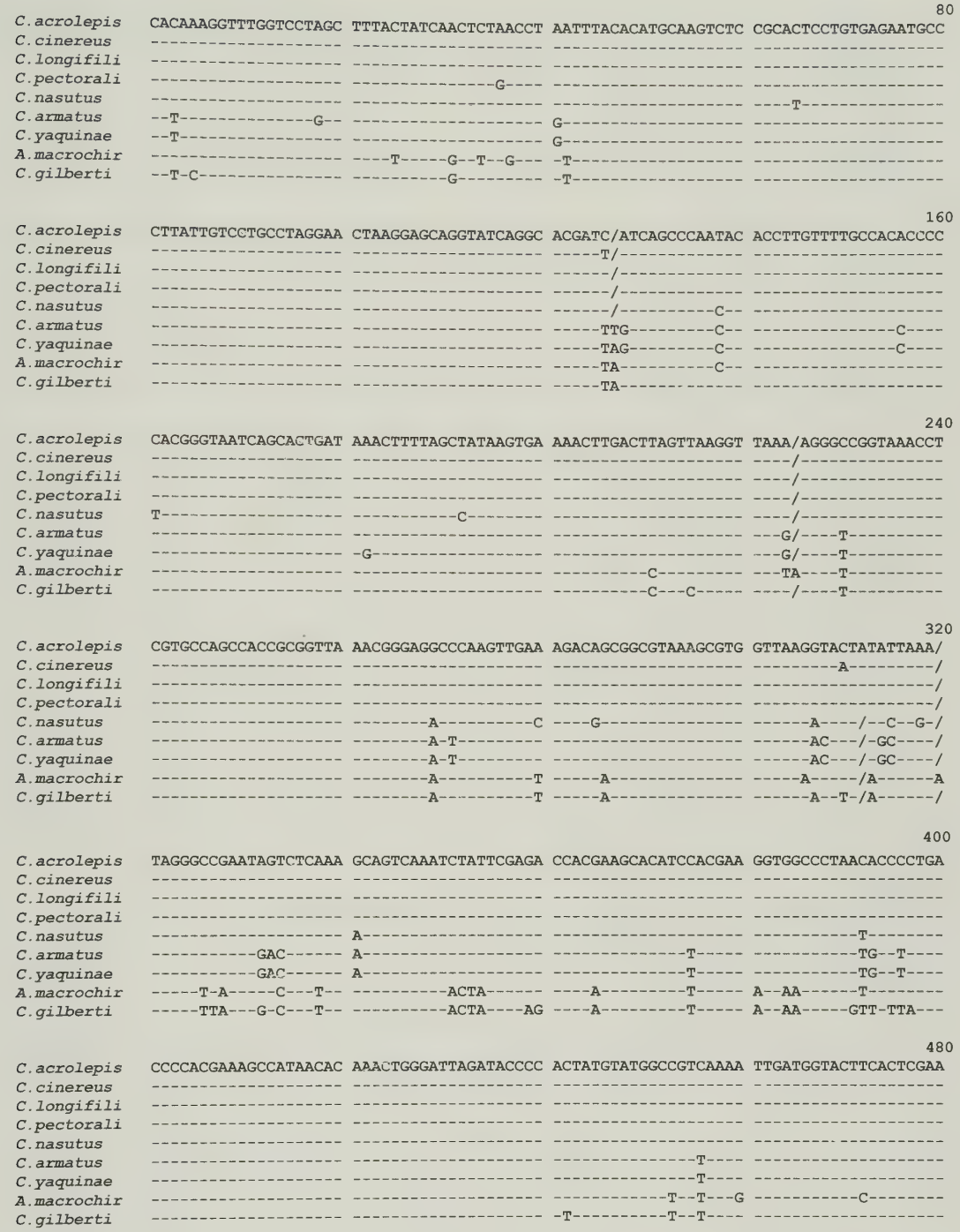

Fig. 4. Comparison of partial nucleotide sequences of the 12S rRNA gene of Coryphaenoides species. A dash indicates an identical nucleotide with that of *C. acrolepis*. The sequences correspond to positions 68-892 of the Atlantic cod homolog (Steinar and Ingrid, 1996). The sequences of Coryphaenoides species are available from the DDBJ/EMBL/GenBank nucleotide databases with accession numbers AB018224-AB018232.

```
                                                                                                                560
C.acrolepis   CCATCCGCCCGGGGACTACG AGCCCTAGCTTAAAACCCAA AGGACCTGGCGGTGCTTTAG ATCCCCCTAGAGGAGCCTGT
C.cinereus    -------------------- -------------------- -------------------- --------------------
C.longifili   -------------------- -------------------- -------------------- --------------------
C.pectorali   -------------------- -------------------- -------------------- --------------------
C.nasutus     ----T--------------A -------------------- -------------------- --------------------
C.armatus     ---------A---A-T---- G----C--------------- -------------------- --------------------
C.yaquinae    ----T----A---A-T---- G----C--------------- -------------------- --------------------
A.macrochir   -------------A------ -----C--------------- -------------------- --------------------
C.gilberti    -------------A------ ----TC--------------- -------------------- --------------------

                                                                                                                640
C.acrolepis   CCTGTAACTGACAACCCCCG TTAAACCTCACCACCCCTTG TCAATCCCGCCTATATACCA CCGTCGTAAGCTTACCCTAT
C.cinereus    ---A---------------- -------------------- -------------------- --------------------
C.longifili   ---A---------------- -------------------- -------------------- --------------------
C.pectorali   ---A---------------- -------------------- -------------------- --------------------
C.nasutus     ---A---------------- ----------------T--- -------------------- --------------------
C.armatus     ---A---------------- --G----------------- -----T----T--------- --------------------
C.yaquinae    ---A---------------- --G----------T------ -----T----T--------- --------------------
A.macrochir   T--A----------T----- -------------------- ----AT-------------- -------------------C
C.gilberti    T--A----------T----- -------------------- ----AT-------------- -------------------C

                                                                                                                720
C.acrolepis   TGAAGGTTCA/AAAGTAGGC TCTAATAGTAATACTAAAGA CGTCAGGTCGAGGTGTAGCG TATGGGGTGGGAAGAGATGG
C.cinereus    ----------/--------- ----------------G--- -------------------- --------------------
C.longifili   ------A-T-/--------- ----------------G--- -------------------- --------------------
C.pectorali   ----------/--------- ----------------G--- -------------------- --------------------
C.nasutus     --------/-A--------- A----------------G--- -------------------- --------------------
C.armatus     --------ATA--------- C----------------G--- -------------------- C------C------------
C.yaquinae    --------ATA--------- C----------------G--- -------------------- C------C------------
A.macrochir   --------/-A------A-- C-C----------------- -------------------- -----A---------A----
C.gilberti    --------/-A------A-- C-C-----C----------- -------------------- -----A---------A----

                                                                                                                800
C.acrolepis   GCTACATTCCCTAATGAAGA GAATACGAACGGTAATTTGA AAAAATTACACTGAAGGAGG ATTTAGTAGTAAGGAAGGAG
C.cinereus    -------------------- ------------C------- -------------------- --------------------
C.longifili   -------------------- -------------------- -------------------- --------------------
C.pectorali   -------------------- -------------------- -------------------- --------------------
C.nasutus     ---------T---------- ------------C------- -------------------- --------------------
C.armatus     -------------------- ---C--------CG------ ------C------------- -------------A-----A
C.yaquinae    -------------------- ------------CG------ ------C------------- -------------A-----A
A.macrochir   -----------------G-- ---C-----T----G----- -----C------------- -------------A-----A
C.gilberti    ------------G-A----G ---C-----T----G----- -----C------------- -------------A-----A

                                    829
C.acrolepis   CAGAGTGCCCTATTGAAGAT GGCCCTAAT
C.cinereus    ------------C------- ---------
C.longifili   ------------C------- ---------
C.pectorali   -------------------- ---------
C.nasutus     -------------------- ---------
C.armatus     -------------------- ---------
C.yaquinae    ------------C------- ---------
A.macrochir   T----A---T-GC------- ---------
C.gilberti    T--------T-GC------- ---------
```

Fig. 4. *continued*

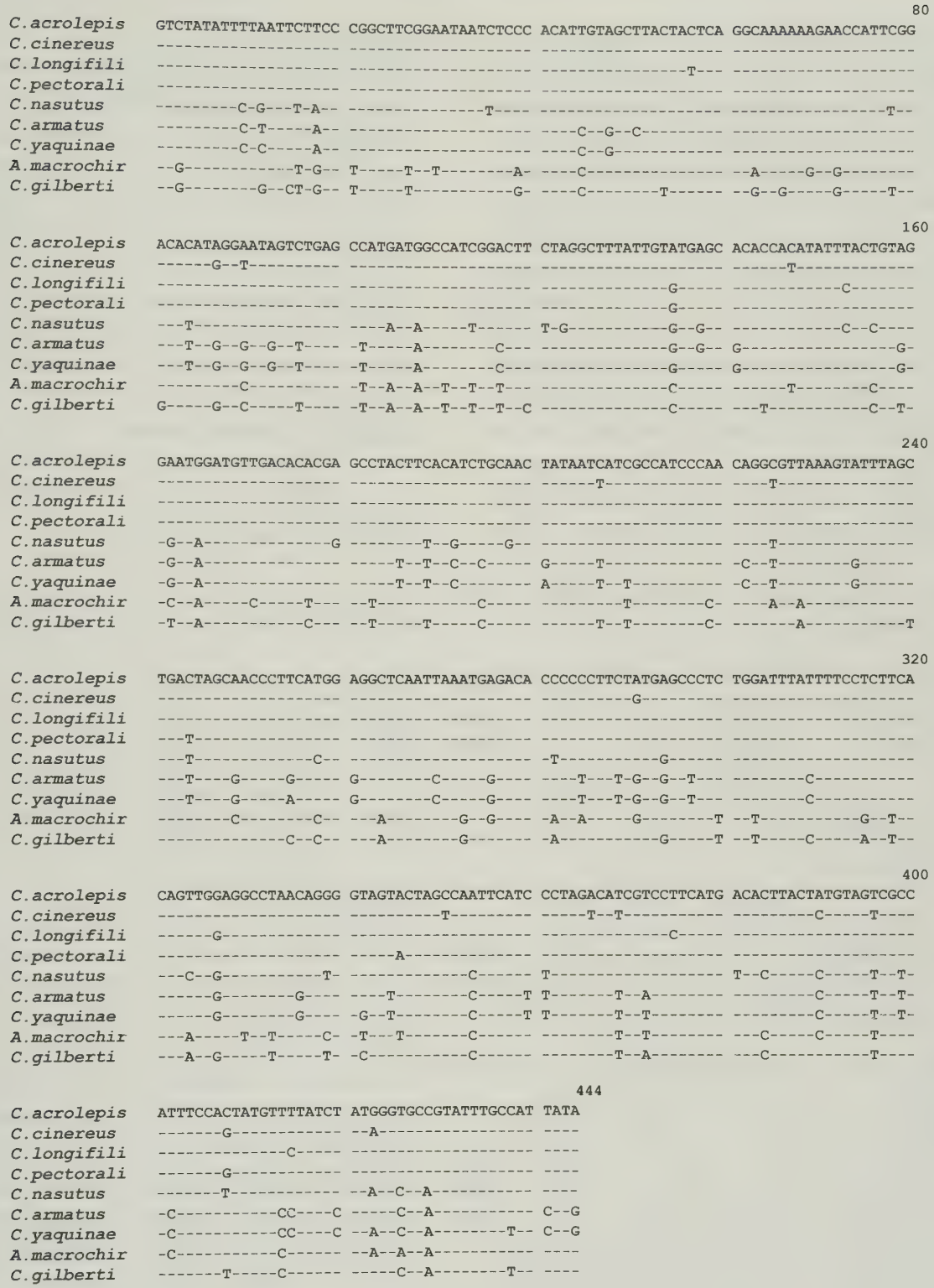

Fig. 5. Comparison of partial nucleotide sequences of the COI gene of *Coryphaenoides* species. A dash indicates an identical nucleotide with that of *C. acrolepis*. The sequences correspond to positions 6167-6611 of the Atlantic cod homolog (Steinar and Ingrid, 1996). The sequences of *Coryphaenoides* species are available from the DDBJ/EMBL/GenBank nucleotide databases with accession numbers AB018233-AB018241.

the transversion of the 12S rRNA gene was not considered to be saturated for these species (Fig. 3 A). Of 829 nucleotides (nt), 114 nt (51 nt in the stem sites and 63 nt in the loop sites) were variable and 79 nt were parsimony-informative (36 nt in stem sites and 43 nt in loop sites). The MP analysis of the 12S rRNA gene produced a most parsimonious length tree with length = 137, consistency index = 0.891, retention index = 0.879, and rescaled consistency index = 0.783 (Fig. 6). This indicates that the phylogenetic positions of the two abyssal species such as *C. armatus* and *C. yaquinae* were separated with a large genetic divergence from the other *Coryphaenoides* species. The phylogenetic trees constructed by the NJ method and the ML method (Ln likelihood = -1874.88) were very similar to the MP tree (Fig. 6).

The COI gene of *Coryphaenoides* species in the region corresponding to positions 6167 to 6611 of the Atlantic cod homolog (Steinar and Ingrid, 1996), was also subjected to phylogenetic analysis. The base composition was A = 25.5%, T = 30.9%, C = 24.4% and G = 19.2%. Nucleotide pairwise distances of the COI gene were greater than those of the 12S rRNA gene and ranged from 0.0090 to 0.1374 (mean = 0.0901) within *Coryphaenoides* and from 0.1374 to 0.1846 (mean = 0.1842) in comparison with the outgroup (Table 3).

Among 444 nucleotide sequences in *Coryphaenoides* species, 117 nucleotide positions were variable and 87 positions were parsimony-informative. The percentage of variable positions in 444 nt of the

Table 2. Pairwise sequence estimates (above diagonal) and number of base substitutions (below diagonal) for 12S rRNA gene

	1	2	3	4	5	6	7	8	9
1. C. armatus		0.0073	0.0571	0.0557	0.0545	0.0570	0.0556	0.0942	0.0885
2 .C. yaquinae	8 (1)		0.0571	0.0584	0.0545	0.0597	0.0556	0.0970	0.0913
3. C. longifilis	45 (7)	45 (7)		0.0049	0.0061	0.0061	0.0286	0.0916	0.0776
4. C. pectoralis	44 (6)	46 (6)	4 (1)		0.0061	0.0037	0.0274	0.0929	0.0789
5. C. cinereus	42 (6)	44 (8)	5 (2)	5 (1)		0.0073	0.0286	0.0902	0.0776
6. C. acrolepis	45 (6)	47 (6)	5 (1)	3 (0)	6 (1)		0.0286	0.0915	0.0775
7. C. nasutus	44 (4)	44 (4)	23 (7)	22 (6)	23 (7)	23 (6)		0.1024	0.0883
8. C. gilberti*	73 (15)	74 (14)	70 (15)	61 (14)	70 (16)	70 (14)	78 (12)		0.0350
9. A. macrochir*	69 (13)	70 (12)	61 (14)	62 (13)	62 (15)	61 (13)	68 (10)	28 (6)	

Above diagonals include pairwise sequence divergenece estimated by the Kimura's two-parameter model. Below diagonals include the number of total base substitutions and that of transversion in parentheses. Asterisks indicate the outgroup species.

Table 3. Pairwise sequence estimates (above diagonal) and number of base substitutions (below diagonal) for COI gene

	1	2	3	4	5	6	7	8	9
1. C. armatus		0.0252	0.1519	0.1525	0.1469	0.1519	0.1488	0.2167	0.2256
2 .C. yaquinae	11 (1)		0.1543	0.1550	0.1438	0.1543	0.1512	0.2141	0.2156
3. C. longifilis	60 (10)	61 (9)		0.0183	0.0442	0.0136	0.0991	0.1822	0.1715
4. C. pectoralis	60 (12)	91 (11)	8 (2)		0.0347	0.0091	0.0969	0.1801	0.1634
5. C. cinereus	58 (12)	57 (11)	19 (2)	15 (2)		0.0300	0.1099	0.1822	0.1540
6. C. acrolepis	60 (10)	61 (9)	6 (0)	4 (2)	13 (2)		0.0991	0.1733	0.1569
7. C. nasutus	59 (9)	60 (8)	41 (3)	40 (5)	45 (5)	41 (3)		0.1664	0.1763
8. C. gilberti*	80 (24)	79 (25)	69 (22)	68 (24)	69 (22)	66 (22)	64 (19)		0.1051
9. A. macrochir*	82 (29)	79 (28)	65 (25)	62 (27)	59 (25)	60 (25)	67 (22)	43 (7)	

Above diagonals include pairwise sequence divergenece estimated by the Kimura's two-parameter model. Below diagonals include the number of total base substitutions and that of transversion in parentheses. Asterisks indicate the outgroup species.

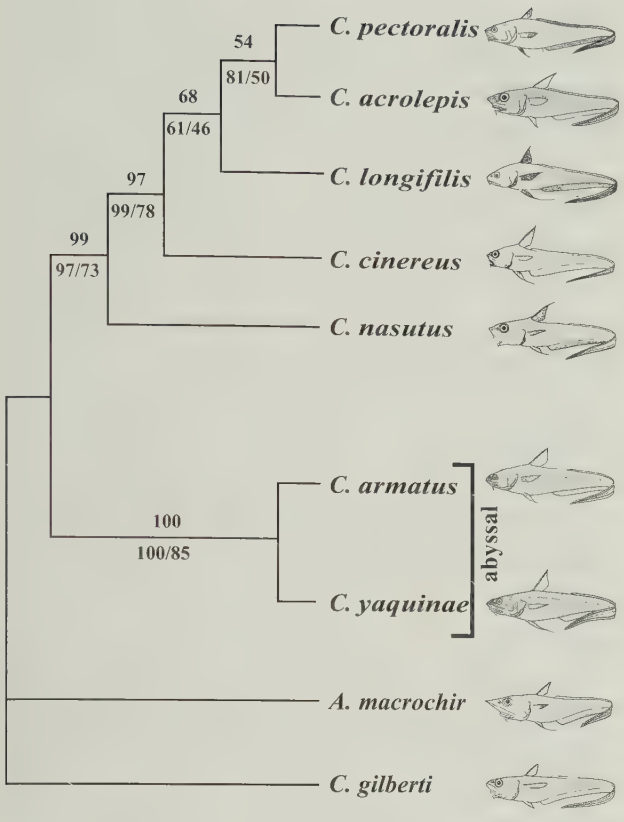

Fig. 6. Phylogenetic tree constructed by the maximum parsimony method based on the 12S rRNA gene of *Coryphaenoides* species. Numbers denote the bootstrap percentages out of 1000 replicates, above branches for MP, left below branches for NJ and right for ML. *A. macrochir* and *C. gilberti* are used as the outgroup. Fish appearances were cited from Nakabo (2000).

COI gene (26.6%) was higher than that in 829 nt of the 12S rRNA gene (13.8%). The TS/TV ratio among *Coryphaenoides* species and outgroup species ranged from 1.0 to 10.0 except for *C. longifilis* and *C. acrolepis* having TV = 0. The TS/TV plot for the COI gene suggested that transitions at the third codon position among the ingroup and outgroup had reached saturation (Fig. 3 B), but not at the first codon position. Of 117 variable nucleotide positions, first and third codon positions accounted for 6.8% and 93.2%, respectively. The MP analysis of the COI gene showed three most parsimonious trees with length = 189, consistency index = 0.778, retention index = 0.722, rescaled consistency index = 0.561, indicating differences among three species, *C. pectoralis*, *C. longifilis* and *C. acrolepis* (Fig. 7 A).

The phylogenetic tree constructed by the NJ method was similar to that by the MP method except that the MP method placed *C. nasutus* in the most basal position of *Coryphaenoides* (Fig. 7 B). The tree based on the COI gene by the NJ method was very similar to that of 12S rRNA gene. The phylogenetic tree constructed by the ML method (Ln likelihood = -1485.98) was similar to one of three

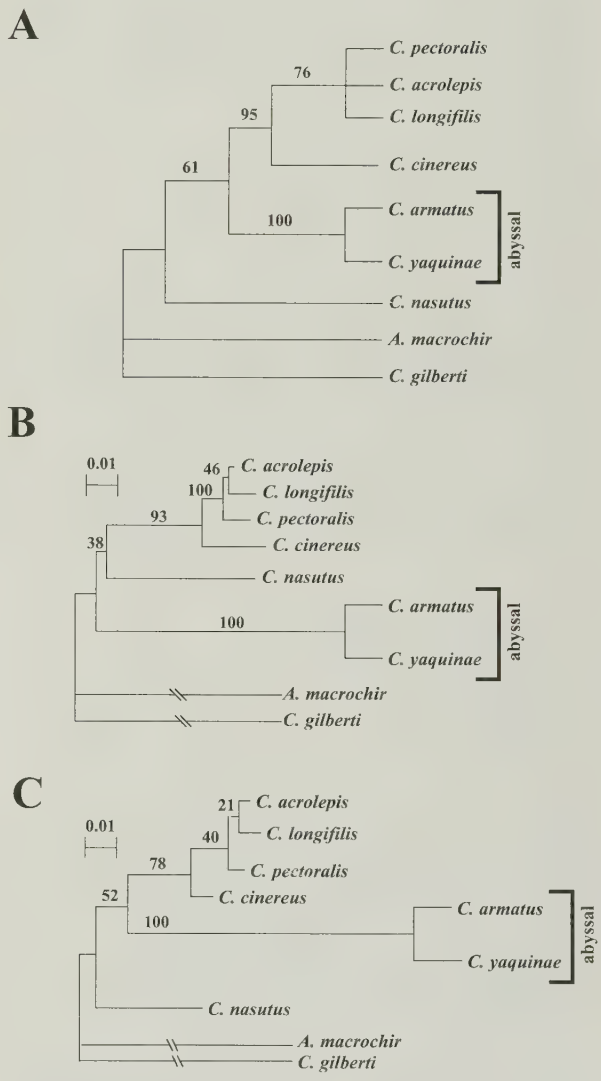

Fig. 7. Phylogenetic trees constructed by maximum parsimony method (A), neighbor joining method (B) and maximum likelihood method (C) based on the COI gene sequences of *Coryphaenoides* species. Numbers on internal branches denotes the bootstrap percentage out of 1000 replicates. Scales indicate the evolutionary distances of the base substitution per site, estimated by the Kimura's two-parameter method. *A. macrochir* and *C. gilberti* are used as the outgroup.

trees by the MP method (Fig. 7 C). Because of the difference in the position of *C. nasutus* among three phylogenetic trees and low bootstrap values for the clade for *C. nasutus* based on the COI gene, the author attempted to reconstruct these phylogenetic tree from data consisting of 1273 bp by combining the sequences of the 12S rRNA (829 bp) and COI (444 bp) genes referring to the previous studies which combined sequence data from different regions of mtDNA (Dutton *et al.*, 1996; Harasewych *et al.*, 1997; Krajewski *et al.*, 1997; Vogler and Welsh, 1997). Fig. 8 shows phylogenetic trees constructed by the MP, NJ and ML methods using combined data of the 12S rRNA and COI genes. The MP method with the combined data produced a most parsimonious tree with length = 329, consistency index = 0.818, retention index = 0.782, rescaled consistency index = 0.639. The trees by the NJ and ML methods (Ln likelihood = -3426.05) with the combined data were the same as constructed by the MP method. The phylogenetic tree based on the combined data was also the same as that based on the 12S rRNA gene. It was noted that the bootstrap values in both the MP and NJ methods based on the combined data were over 60% for every clade and higher than those only on the 12S rRNA gene. Therefore, the phylogenetic tree based on combined data from 12S rRNA and COI genes is considered to be most reliable for the taxonomic position of each *Coryphaenoides* species. Two species such as *C. armatus* and *C. yaquinae*, which are known as abyssal species, were placed at the most basal position in *Coryphaenoides* species, indicating that abyssal species spread into the deep sea earlier than the radiation of other *Coryphaenoides*. The trees imply that five non-abyssal species hereafter diverged successively in the order of *C. nasutus*, *C. cinereus*, *C. longifilis*, *C. acrolepis* and *C. pectorolis*.

Discussion

The present study clearly demonstrated reliable molecular phylogenetic trees of seven *Coryphaenoides* species based on mtDNA analysis and their radiation process into the deep sea. The author's finding proposes new taxonomic positions of *Coryphaenoides* species, while significantly differ from previous taxonic relationships based on morphological (Iwamoto and Sazonov, 1988) and biochemical (Wilson *et al.*, 1991; Wilson, 1994) characters. One previous result defining the subgenera was not complete (Iwamoto and Sazonov, 1988). Of *Coryphaenoides* species used in this study, *C. nasutus*, *C. cinereus*, and *C. acrolepis* were included in the subgenus *Coryphaenoides*, whereas *C. armatus*, *C. yaquinae*, *C. pectoralis* and *C. longifilis* were in the subgenus *Nematonurus*.

Wilson (1994) suggested by biochemical studies on LDH isoenzymes that *Coryphaenoides* species diverged on upper continental slope and subsequently spread into the abyssal ocean. However, the phylogenetic trees in the present study placed the abyssal species such *C. armatus* and *C. yaquinae* at the most basal position. This result indicates that the abyssal group separated from non-abyssal one on the upper continental slopes before the radiation of non-abyssal species, and that nonabyssal species thereafter were diverged on the upper slopes. Miya and Nishida (1996) reconstructed the phylogenetic tree of the midwater deep-sea fish genus *Cyclothone* based on mtDNA, showing that *Cyclothone* communities in different localities are of polyphyletic assemblages. The molecular phylogenetic tree of *Cyclothone* placed bathypelagic *Cyclothone* species such as C. obscura and C. parapallida at the most basal position, as well as the tree of *Coryphaenoides* found in this study. It has been believed that new species appear when the gene flow between different populations is interrupted (France and Kocher, 1996). The deep-sea environment is considered to enhance the speciation process by forming a barrier of the gene flow even in the absence of absolute geographic isolation. Hydrostatic pressures have various effects on biochemical and physiological processes (Siebenaller and Somero, 1978, 1989; Siebenaller, 1991; Childress and Thuesen, 1995; Gibbs, 1995; Siebenaller and Murray, 1995). Such marine environment would be one of important factors in the speciation process. The present molecular phylogenetic tree indicates that abyssal *Coryphaenoides* species diverged from non-abyssal species as the first event of *Coryphaenoides*

speciation. The tree also suggests that a new function, which enables species to adapt to high hydrostatic pressures, was provided to proteins by a very few amino acid substitutions rather than by gradual accumulation of successive mutations.

The present phylogenetic trees of *Coryphaenoides* showed that three species, *C. pectoralis*, *C. longifilis* and *C. acrolepis*, are very closely related with their clades having relatively low bootstrap values. The taxonomic position of *C. pectoralis* has long been disputed and this species was recently proposed to be placed in a separate genus Albatrossia (Iwamoto and Sazonov, 1988). However, both NJ and MP analyses of the mitochondrial 12S rRNA and COI genes in the present study indicated that the taxonomic position of *C. pectoralis* is placed within the genus *Coryphaenoides* as in the case of the result of isoenzyme analysis (Wilson *et al.*, 1991; Wilson, 1994). The low bootstrap values were attributed to the small sequence divergence among the three species, 0.0049-0.0061 in the 12S rRNA gene and 0.0180-0.0135 in the COI gene. In order to ascertain the relationship among these species, the analysis of the mitochondrial control region would be more suitable because this analysis has been used for previous studies on closely related species and populations due to its high nucleotide substitution rate (Meyer *et al.*, 1990; Fajen and Breden, 1992; Sturmbauer and Meyer, 1992).

The present findings show that abyssal *Coryphaenoides* such as *C. armatus* and *C. yaquinae* diverged from non-abyssal species as the first event of *Coryphaenoides* speciation. This radiation time can be estimated by the mutation rate of mtDNA, although an absolute divergence time from molecular data cannot be calculated because there is no fossil record for *Coryphaenoides*. Although the mutation rate in mtDNA is estimated to be 1-2%/MY, it is known to be influenced by thermal habitat, generation time, and metabolic rate (Martin and Palumbi, 1993; Rand, 1994). Ectotherms have slower mutation rates than endotherms, and the mutation rate of typical ectotherms is estimated to be 0.3-0.7%/MY (Kocher *et al.*, 1989; Martin *et al.*, 1992; Martin and Palumbi, 1993; Rand, 1994). The estimated mutation rate for TV (0.14%/MY) in rRNA genes is known to be about the same both in endotherms and ectotherms (Kraus and Miyamoto, 1991; Caconne *et al.*, 1994; Ritchie *et al.*, 1996). Since the average percentage of sequence divergence only in TV between the abyssal and non-abyssal *Coryphaenoides* was 0.724%, the radiation time between the two groups was calculated to be 5.2 million years ago (Fig. 8). In the case of the COI gene, only the TV rate is applicable to the estimation of the radiation time between the abyssal and non-abyssal groups because TS substitutions in this COI gene were saturated and TV was considered to be accumulating approximately linearly with time (Miyamoto and Boyle, 1989). Since the average percentage of sequence divergence in only TV of the COI gene between the abyssal and non-abyssal *Coryphaenoides* was 2.27%, the radiation time was estimated to be approximately 3.2-7.6 million years ago (Fig. 8). Many fossil deep-sea fish found in the stratum between Miocene and Pliocene times have the same morphology as that of the present deep-sea fish, suggesting the occurrence of the present deep-sea fish in these times (Uyeno, 1967, 1980).

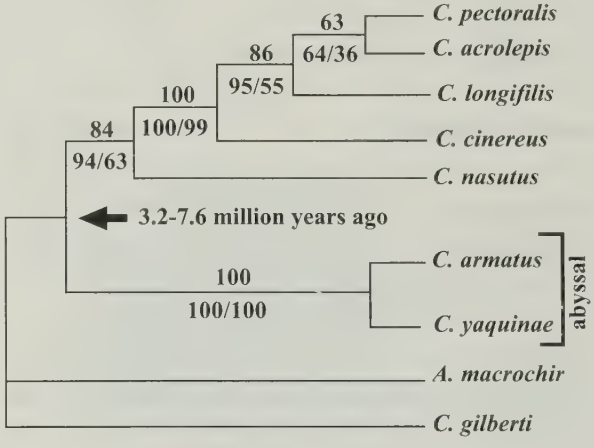

Fig. 8. Phylogenetic tree constructed by the maximum parsimony method by combination data of the 12S rRNA and the COI genes of *Coryphaenoides* species. Numbers denote the bootstrap percentage out of 1000 replicates, above branches for MP, left below branches for NJ and right for ML. *A. macrochir* and *C. gilberti* are used as the outgroup. An arrow indicates the point of the radiation between abyssal and non-abyssal *Coryphaenoides* species, which was estimated to have occured approximately 3.2-7.6 million years ago (see text).

The present study also showed that *Coryphaenoides* arose between Miocene and Pliocene times, about 3.2-7.6 million years ago.

Chapter 2. Characterization and structure of α -skeletal actin of the deep-sea fish

In comparison with studies on thermal biology (Somero, 1995; Hochachka and Somero 2002; Watabe, 2002), those dealing with the effects of pressures on biological functions had been poorly carried out. The development of instruments, however, has accelerated the studies in that area (Mozhaev *et al.*, 1996). Hydrostatic pressures act on the volume changes along biological processes. There are a number of reviews dealing with the effects of hydrostatic pressures (Heremans, 1982; Jaenicke, 1983; Weber and Drickamer, 1983; Silva and Weber, 1993; Mozhaev *et al.*, 1996). The pressure sensitivity of chemical reactions is dependent upon the magnitude of the volume change associated with the equilibrium constant (K)or rate constant (k), according to the following:

$$\delta \ln K / \delta P = -\delta V / RT$$

$$\delta \ln k / \delta P = -\delta V\# / RT$$

where P = pressure, R = the gas constant, T = the absolute temperature and δV = the difference in volume between the final and initial state at the system at equilibrium and $\delta V\#$ = the difference in volume between the transition and ground states. The response of a reaction or step in a reaction to an increase in pressure is governed by the LeChatelier's principle. If a reaction step proceeds with an increase in volume, the increased pressure will inhibit that step. On the other hand, processes accompanied with a decrease in volume will be enhanced by an increase in pressure.

Section 1. Characterization of the deep-sea fish α -actin under high pressures

Actin has a function in polymerization of G-actin to F-actin in neutral salts. While the volume is increased following polymerization, the reaction is strongly perturbed by high pressures (Ikkai and Ooi, 1966). It is noteworthy that δV, which is associated with polymerization of α-actin in the preparations from abyssal fish *C. armatus*, was much smaller, which is advantageous for a deep-sea habitat, than that from non-abyssal fish *C. acrolepis* (Swezey and Somero, 1985). Actin is a highly conserved protein found in eukaryotic cells; α-actin in carp and that in rat have 99.4% homology at the amino acid sequence level (Collins and Elzinga, 1975; Watabe *et al.*, 1995). It is therefore surprising that differences in δV with polymerization of this highly conserved protein have been found between two species of *Corypanenoides* that inhabit different niches.

In this section, the author investigated the polymerization kinetics at high pressures of α -skeletal actin from two abyssal species (*C. armatus* and *C. yaquinae*), non-abyssal species (*C. acrolepis*), carp and chicken.

Materials and Methods

Materials

C. acrolepis specimens (habitat depth 180-2000m) were collected using large deep-sea pots by R/V Soyo-maru of the National Research Institute of Fisheries Science. Sampling locations were 41-40.20' N and 142-57.40' E at the depth of 180 m. *C. armatus* (habitat depth 2700-5000m)and *C. yaquinae* (habitat depth 4000-6400m)were collected as described in Chapter 1. Non-abyssal species, *C. acrolepis*, was selected as a sample fish based on the molecular phylogenetic tree in Chapter 1 and on the amino acid sequences in Section 2 in this chapter. Carp and chicken samples were purchased from local suppliers. All samples were stored below -80℃ until use.

Actin

Actin was isolated from the fast skeletal muscle of each species according to Spudich and Watt (1971) and purified by gel-filtration chromatography on a Sephadex G-200 column (2.5cm × 100cm) in G-buffer (0.2mM ATP, 0.2mM $CaCl_2$, 0.5mM β-mercaptoethanol, 2.0mM Tris-HCl pH 7.8) (MacLean-Fletcher and Pollard, 1980). Actins were stored in G-buffer at 4℃ after purification and used within 3 days. Mg^{2+}-G-actin converted

from Ca^{2+}-G-actin as described previously (Chen and Rubenstein, 1995; Pollard, 1986) was used immediately after the conversion. The concentration of G-actin was determined spectrophotometrically using an absorption coefficient of 0.63mL/mg at 290nm (Houk and Ue, 1974).

Polymerization, critical concentration and δV in assembly of G-actin into F-actin

Polymerization of Mg^{2+}-G-actin was initiated by adding KCl and $MgCl_2$ to a final concentration of 50 and 2 mM, respectively. Polymerization was monitored by light scattering at 4℃, with excitation and emission wavelengths both set at 400nm in a high-pressure cell with a high-pressure pump (PCI-400 cell and TP-500 pump Teramecs) (Fig. 9). The change in light scattering was recorded as a function of time. The critical concentration was determined as described previously (Tobacman *et al.*, 1983; Chen *et al.*, 1993). The volume change(δV) in assembly of G-actin into F-actin was calculated by the method of Swezey and Somero (1985).

Results

Actin polymerization and volume change

The author measured the polymerization kinetics of α-actin isolated from each species as a function of pressure at various actin concentrations by monitoring light scattering assay. Actin prepared from all species except carp underwent polymerization up to 60MPa, the maximum pressure in the present study. However, carp actin polymerized only at pressures of 20 MPa or less. The half-time to steady state in polymerization and critical concentrations were determined from the polymerization kinetics (Fig. 10). The half-times for all actins increased with high pressures, and at atmospheric pressure the polymerization half-time was shortest for carp actin. The half-times for chicken and non-abyssal actin species increased markedly above 20 MPa, and were about 5.6-and 7.3-fold longer, respectively, at 60 Mpa than those at atmospheric pressure (Fig. 10 A). By contrast, the half-time of actin polymerization for the two abyssal species had increased only about 2.7-fold at 60 MPa in the same comparison as above.

The critical concentrations of actin also increased with high pressure for each species (Fig. 10 B). The critical concentrations of the two abyssal actins were higher than those of other species at 20MPa and lower pressures, and increased slightly from 0.1 to 60MPa.

δV associated with polymerization at each pressure was determined from the respective critical concentrations (Fig. 10 C). The two abyssal actin species had a much smaller δV at each pressure than did the other species, in agreement with previous reports (Swezey and Somero, 1985). δV of actins from chicken and non-abyssal species

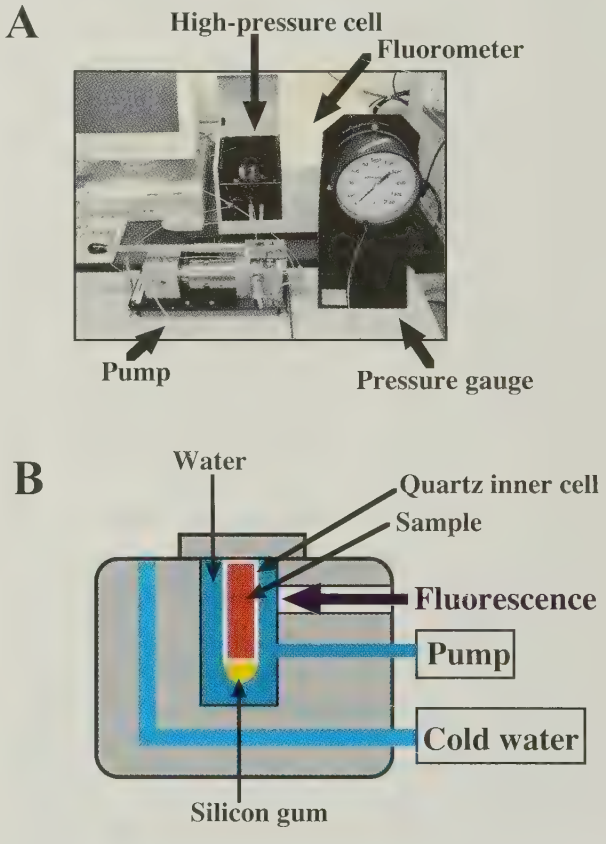

Fig. 9. High-pressure measurement system. (A) Component of high-pressure measurement system. A high-pressure cell is set in a fluorometer. High-pressure is generated with a hand pump. (B) A high-pressure cell. A sample is put in the quartz inner cell. High-pressure generated with the pump is conveyed by the water medium. The water medium pushes up the silicon gum and the sample in the inner cell is compressed. The whole cell is cooled with cold water.

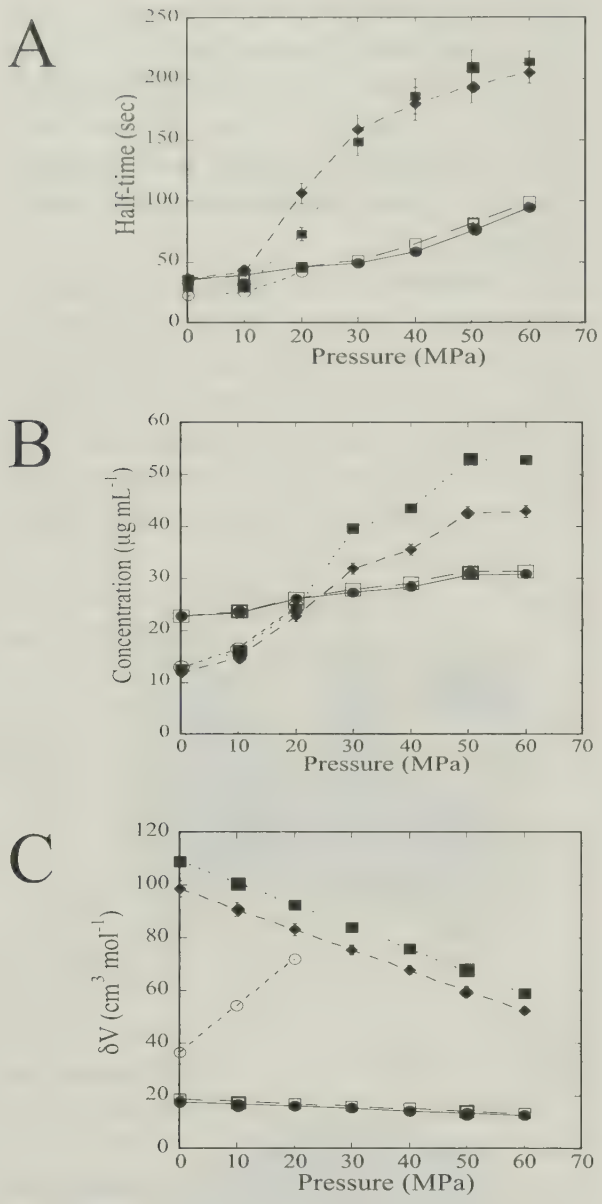

Fig. 10. Effects of pressure on actin polymerization. (A)Half-time to steady state in polymerization of actin. (B)Critical concentration of actin at various pressures. (C)Volume change(δ V)associated with polymerization of actin at various pressures. Symbols are as follows: *C. yaquinae*(●); *C. armatus* (□); Chicken(◆); carp(○); C. acrolepis(■). Values are means ± S.D. of four independent experiments. Errors for several experiments are too small to show error bars.

decreased with the increase of pressure, whereas those from the abyssal species showed little variation.

These observations indicate that the coefficient of compressibility for F-actin was larger than that for G-actin for chicken and non-abyssal species, and that the space produced by actin-actin interactions was reduced by high pressures. Clearly, there was no such reduction in space for actins from the abyssal species. Unexpectedly, δ V of carp actin, unlike those of other species, increased with the increase of pressure, indicating that the coefficient of compressibility for G-actin was larger than that for F-actin from carp. In other words, carp G-actin was softer than those of other species. Thus, this would explain why carp actin was able to polymerize at pressures of only 20 MPa or less.

Section 2. Molecular cloning of α-actin from the deep-sea fish *Coryphaenoides*

In Section 1 of this chapter, the author demonstrated that α-actin prepared from two abyssal *Coryphaenoides* species are more pressure insenstive and have smaller δ V of assembly than those from non-abyssal *Coryphaenoides* species, carp and chicken. Actin is a highly conserved protein; the α-actin in carp and that in rat have 99.4% homology at the amino acid sequence level (Collins and Elzinga, 1975; Watabe *et al.*, 1995). This high homology suggests that a few amino acid substitutions would cause much smaller δ V of α-actin from the deep-sea fish.

The objective of this section is to isolate α-actin cDNA clones from the fast skeletal muscle of abyssal and non-abyssal *Coryphaenoides* and identify the amino acid residues making α-actin of the abyssal species adaptive to high hydrostatic pressure.

Materials and Methods

Materials

Samples were collected as described in Chapter 1 and stored frozen after collection. *C. acrolepis* and *C. cinereus* were selected as non-abyssal species based on the results of the molecular phylogenetic analysis in Chapter 1.

Isolation of α-actin cDNA from *C. acrolepis*, *C. armatus* and *C. yaquinae*

Total RNA was extracted from the dorsal fast

skeletal muscles of *C. acrolepis*, *C. armatus* and *C. yaquinae* with RNA extract solution according to the manufacturer's protocol (Isogen; Nippon Gene). Poly (A)+mRNA was isolated from the total RNAs using Oligotex-dT30 <Super>(Takara). About 5g of poly (A)+ mRNA was used to synthesize double-stranded cDNA with a cDNA synthesis kit (Pharmacia Biotech) with the Not-1 d(T) 18primer. The skeletal muscle cDNA libraries were constructed using the synthesized cDNAs and a phage vector λ ZAPII according to the manufacturer's instructions (Stratagene). The α-actin probes were prepared by polymerase chain reaction(PCR). The 5' primer was synthesized referring to the DNA sequences of α-actins from various animals, and corresponded to positions from 372 to 392 of carp α-actin cDNA (Watabe, 1995). The nucleotide sequence of the PCR primers were 5'-CATGTTTGAGACCTTCA ACGT-3' for 5' primer-1 and 5'-TGGAAGAATTCG CGGCCGCA-3' (Not-1 primer) for 3' primer. The conditions of PCR amplification were 30cycles of 94℃ for 30sec, 5℃ for 30sec, and 72℃ for 2min, using a DNA Thermal Cycler Model 2400 (Perkin Elmer) . The reaction mixture (100 μL) contained 2mM $MgCl_2$, 200mM dNTP, 100pmol of each primer, 2.5units of Taq DNA polymerase, and 50 ng of the cDNA. From these PCR amplifications, the author obtained two PCR products differing in the length from each other. The nucleotide sequences of these PCR products subcloned in a T-vector (Novagen) were homologous to that of the carp α-actin cDNA (Watabe *et al.*, 1995). Therefore, the author synthesized two specific primers that corresponded to the region of 1141 to 1160 nt of *C. acrolepis* α -actin cDNA (Fig. 11), 5' primer-2: 5'-AACATTTG TCTCCATCATTT-3 for α-actin 1, and 5' primer-3: 5'-CACCCAGCGTCTGCTCTCAG-3' for α-actin 2 (Fig. 11) , according to these 3'-noncoding regions. PCR amplification was performed under the same conditions described above with Not-1 primer plus 5' primer-2 or 5' primer-3 and using the cDNA libraries. These DNA fragments were labeled with a DIG DNA labeling kit according to the manufacturer's instructions (Boehringer Mannheim) and used as the probes for screening the cDNA library and Northern blot analysis. DNA sequencing was performed with Dye Deoxy terminator cycle sequencing kit using a Model 373A DNA Sequencer (Applied Biosystems).

Isolation of α-actin cDNA from *C. cinereus*

To isolate α-actin cDNAs from *C. cinereus*, reverse transcription (RT)-PCR was performed with sense primers that were synthesized referring to nucleotide sequences in the region of -30 to -11 of the two α-actin cDNA sequences of *C. acrolepis*. The 5' primer-4 for α-actin 1 of 5'-AGCCGCAGACACTC ACCTAA-3' or 5' primer-5 for α-actin 2 of 5'-CCGG AGCTACCAACTGAATA-3' (Fig. 11) was used for PCR with the Not-1 primer as the antisense primer encoding the 3'-end. Single-strand cDNA, which involved the Not-1 primer sequence at the 5'-end, was synthesized from 5 μg of total RNA which had been extracted from the dorsal fast skeletal muscle of *C. cinereus* using the T-primed First-strand kit with the Not-1 d $(T)_{18}$ primer (Pharmacia Biotech). One-fourth of the resultant cDNA was used as a template for PCR, the reaction mixture of which (100 μL) contained 2mM $MgCl_2$, 200mM dNTP, 100pmol of each primer and 2.5units of Taq DNA polymerase. The conditions for PCR were 40 cycles of 94℃ for 1 min, 60℃ for 1 min, and 72℃ for 2 min, using a DNA Thermal Cycler Model 2400 (Perkin Elmer). The two PCR products were subcloned into a T-vector (Novagen). DNA sequencing was performed with Dye Deoxy terminator cycle sequencing kit using a Model 373A DNA Sequencer (Applied Biosystems).

Northern blot analysis

Total RNA was extracted from the dorsal fast skeletal muscle of *C. acrolepis*, and Northern blot analysis was performed using the total RNA (15 μg per lane). RNA was size-fractionated on a 1.2% agarose gel containing 50% formamide by electrophoresis, and then transferred to a nylon membrane (Amersham Bioscience). The probes that had been used for screening of cDNA library were used as the hybridization probes. Hybridization and detection were performed according to the manufacturer's instructions (Boehringer Mannheim).

```
                -49
C.acrolepis-1   TTGGGTCTT TCTCTCTCCA                          GAAAGCCATC
C.acrolepis-2a    TTAC... GT.....GAT                             ...T..A.
C.cinereus-1                                                    G........
C.cinereus-2a                                                     ..T..A.
C.armatus-2a                        T CC..CGGAG. TAC..A..G. AT...T..A.
C.armatus-2b                     ..... ......... ......... .G.....T..
C.yaquinae-2a                       T CC..CGGAG. TAC..A..G. AT...T..A.
C.yaquinae-2b                    ..... ......... ......... .G.....T..

                START                                                   50
C.acrolepis-1   ATGTGTGACG ATGAGGAGAC TACCGCCCTT GTGTGCGACA ACGGCTCCGG
C.acrolepis-2a  .......... .C..A..... C........C .......... ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .......... .C..A..... C........C .......... ..........
C.armatus-2a    .......... .C..A..... C........C .......... ..........
C.armatus-2b    .......... .......... .......... .......... ..........
C.yaquinae-2a   .......... .C..A..... C........C .......... ..........
C.yaquinae-2b   .......... .......... .......... .......... ..........

                                                                   100
C.acrolepis-1   CCTGGTGAAG GCTGGGTTCG CCGGAGACGA TGCCCCCAGG GCTGTCTTCC
C.acrolepis-2a  ...C...... .....C.... .......... C..A...... ..C.......
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ...C...... .....C.... .......... C..A...... ..C.......
C.armatus-2a    ...C...... .....C.... .......... C..A...... ..C.......
C.armatus-2b    ...C...... .......... .......T.. C..G...... ..........
C.yaquinae-2a   ...C...... .....C.... .......... C..A...... ..C.......
C.yaquinae-2b   ...C...... .......... .......T.. C..G...... ..........

                                                                   150
C.acrolepis-1   CCTCCATCGT CGGCCGCCCC CGTCACCAGG GTGTCATGGT CGGTATGGGT
C.acrolepis-2a  .A........ .........T .......... .C........ G..C.....G
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .A........ .........T .......... .C........ G..C.....G
C.armatus-2a    .A........ .........T .......... .C........ G..C.....G
C.armatus-2b    .A........ .......... .......... .......... A..C.....G
C.yaquinae-2a   .A........ .........T .......... .C........ G..C.....G
C.yaquinae-2b   .A........ .......... .......... .......... A..C.....G

                                                                   200
C.acrolepis-1   CAGAAAGACT CCTACGTCGG CGACGAGGCC CAGAGCAAGA GAGGTATCTT
C.acrolepis-2a  .....G.... .......G.. .......... .......... ........C.
C.cinereus-1    .....G.... .......... .......... .......... ..........
C.cinereus-2a   .....G.... .......G.. .......... .......... .G......C.
C.armatus-2a    .....G.... .......G.. .......... .......... .G..C...C.
C.armatus-2b    .....G.... ......CG.. .......... .......... .G......C.
C.yaquinae-2a   .....G.... .......G.. .......... .......... .G..C...C.
C.yaquinae-2b   .....G.... .......G.. .......... ........A. .G......C.

                                                                   250
C.acrolepis-1   GACTCTTAAG TACCCCATTG AGCACGGCAT CATCACCAAC TGGGACGACA
C.acrolepis-2a  ...C..C... ........C. .......... .......... ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ...C..C... ........C. .......... .......... ..........
C.armatus-2a    ...C..C... ........C. .......... .......... ..........
C.armatus-2b    ...C..C... .......... .......... .........T ..........
C.yaquinae-2a   ...C..C... ........C. .......... .......... ..........
C.yaquinae-2b   ...C.CC... .......... .......... .........T ..........
```

Fig. 11. Comparison of cDNA nucleotide sequences encoding α-actin 1, α-actin 2a and α-actin 2b. The dot indicates an identical nucleotide with that of *C. acrolepis* α-actin 1. 5' primer-2, 3, 5' primer-4, 5 and polyadenylation signals are indicated by red, blue and boldfaced letters, respectively. These sequences are available from the DDBJ/EMBL/GenBank databases. The accession numbers are AB021649, AB021650, AB021651, AB021652, AB086240, AB086241, AB086242, and AB086242 from the top.

```
                                                                                                300
C.acrolepis-1   TGGAGAAGAT CTGGCACCAC ACCTTCTACA ATGAGCTGCG CGTGGCCCCC
C.acrolepis-2a  .......... .......... .......... .C........ T.........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .......... .......... .......... .C........ T.........
C.armatus-2a    .......... .......... .......... .C........ T.........
C.armatus-2b    .......... .......... .......... .C........ ..........
C.yaquinae-2a   .......... .......... .......... .C........ T.........
C.yaquinae-2b   .......... .......... .......... .C........ ..........

                                                                                                350
C.acrolepis-1   GAGGAGCACC CCACCCTGCT CACTGAGGCC CCCCTGAACC CCAAGGCTAA
C.acrolepis-2a  .......... .......... ...C...... .....C.... .......C..
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .......... .......... ...C...... .....T.... .......C..
C.armatus-2a    .......... .....T.... ...C...... .....C.... .......C..
C.armatus-2b    .......... .......... .......... .....C.... ..........
C.yaquinae-2a   .......... .......... ...C...... .....C.... .......C..
C.yaquinae-2b   .......... .......... .......... .....C.... ..........

                                                                                                400
C.acrolepis-1   CCGTGAAAAG ATGACCCAGA TCATGTTTGA GACCTTCAAC GTCCCCGCCA
C.acrolepis-2a  .A.G..G... .......... .......C.. .......... ..G.......
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .A.G..G... .......... .......C.. .......... ..G.......
C.armatus-2a    .A.G..G... .......... .......C.. .......... ..G.......
C.armatus-2b    .A....G... .....A.... .......... .......... ..G.......
C.yaquinae-2a   .A.G..G... .......... .......C.. .......... ..G.......
C.yaquinae-2b   .A....G... .....A.... .......... .......... ..G.......

                                                                                                450
C.acrolepis-1   TGTATGTGGC CATCCAGGCT GTGCTGTCCC TGTACGCTTC CGGCCGTACC
C.acrolepis-2a  ....C..... .........C .......... .......C.. ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ....C..... .........C .......... .......C.. ..........
C.armatus-2a    ....C..... .........C .......... .......C.. ..........
C.armatus-2b    .......... ....A....C .......... .......... ......C...
C.yaquinae-2a   ....C..... .........C .......... .......C.. ..........
C.yaquinae-2b   .......C.. ....A....C .......... .......... ...A..C...

                                                                                                500
C.acrolepis-1   ACCGGTATTG TGCTGGATGC TGGTGATGGT GTCACCCACA ACGTCCCAGT
C.acrolepis-2a  ..T.....C. .......CT. C.....C... ..G....... ....G..G..
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ..T.....C. .......CT. C.....C... ..G....... ....G..G..
C.armatus-2a    ..T.....C. .......CT. C.....C... ..G....... ....G..G..
C.armatus-2b    ..T.....C. .......CT. C..G..C... ..G.....T. ....G..G..
C.yaquinae-2a   ..T.....C. .......CT. C.....C... ..G....... ....G..G..
C.yaquinae-2b   ..A.....C. .......CT. C..G..C... ..G.....T. .......C..

                                                                                                550
C.acrolepis-1   GTATGAGGGT TACGCCCTGC CCCACGCCAT CATGCGTCTT GACCTGGCTG
C.acrolepis-2a  .........C .....G.... .......... ......C..G ........C.
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .........C .....G.... .......... ......C..G ........C.
C.armatus-2a    ...C.....C .....G.... .......... ......C..G ........C.
C.armatus-2b    ...C...... .....T.... .......... ....A.G..G ...T....C.
C.yaquinae-2a   ...C.....C .....G.... .......... ......C..G ........C.
C.yaquinae-2b   A..C.....C .....T.... .......... ....A.GT.G ...T....C.

                                                                                                600
C.acrolepis-1   GTCGCGACCT GACCGACTAC CTGATGAAGA TCCTGACCGA GCGTGGCTAC
C.acrolepis-2a  .C........ C......... ..C....... ....C..... ...C..A...
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .C........ C......... ..C....... ....C..... ...C..A...
C.armatus-2a    .C........ C......... ..C....... ....C..... ...C..A...
C.armatus-2b    .......... C......... ..C....... ....C..... .A.G..G...
C.yaquinae-2a   .C........ C......... ..C....... ....C..... ...C..A...
C.yaquinae-2b   ....G..... C..A...... ..C....... .......... .A.G......
```

Fig. 11. *continued*

```
                                                                                                650
C.acrolepis-1   TCTTTCGTTA CCACCGCCGA GCGTGAGATC GTGCGCGACA TCAAGGAGAA
C.acrolepis-2a  ..C.....C. .......... .......... .......... ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ..C.....C. .......... .......... .......... .......A..
C.armatus-2a    ..C.....C. .......... ...G...... .......... ..........
C.armatus-2b    ........C. .......... .......... .......... ..........
C.yaquinae-2a   ..C.....C. .......... ...G...... .......... ..........
C.yaquinae-2b   AG......C. .......... .......... .......... ..........

                                                                                                700
C.acrolepis-1   GCTGTGCTAT GTGGCTCTGG ACTTCGAGAA CGAGATGGCC ACCGCCGCCT
C.acrolepis-2a  .........C ..C..C.... .......... .........A ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .........C ..C..C.... .......... .........A ..........
C.armatus-2a    .........C ..C..C.... .......... .........G ..........
C.armatus-2b    .......... .....G.... .......... .........G ........G.
C.yaquinae-2a   .........C ..C..C.... .......... .........G ..........
C.yaquinae-2b   .......... .....G.... .......... .........G ........T.

                                                                                                750
C.acrolepis-1   CCTCCTCCTC CCTGGAGAAG AGCTACGAGC TTCCCGACGG TCAGGTCATC
C.acrolepis-2a  .......... .......... .....T.... .G........ ..........
C.cinereus-1    .......... .........A .......... .......... ..........
C.cinereus-2a   .......... .......... .......... .G........ ..........
C.armatus-2a    .......... .......... .......... .G........ ..........
C.armatus-2b    .......A.. T......... .......... .G........ ..........
C.yaquinae-2a   .......... .......... .......... .G........ ..........
C.yaquinae-2b   .......A.. .......... ..T....... .G........ ..........

                                                                                                800
C.acrolepis-1   ACCATAGGAA ACGAGCGTTT CCGTTGCCCT GAGACCCTCT TCCAGCCTTC
C.acrolepis-2a  .....C..C. .......G.. ...C.....C .......... .......C..
C.cinereus-1    .....C.... .......... .......... .......... ..........
C.cinereus-2a   .....C..C. .......C.. ...C.....C .......... .......C..
C.armatus-2a    .....C..C. .......C.. ...C.....C .......... .......C..
C.armatus-2b    ........C. .......C.. ...C...... .......... ..........
C.yaquinae-2a   .....C..C. ....A..C.. ...C.....C .......... .......C..
C.yaquinae-2b   ........C. .......C.. ...C...... .......... ..........

                                                                                                850
C.acrolepis-1   CTTCATCGGA ATGGAGTCCG CCGGTATCCA TGAGACCGCC TACAACAGCA
C.acrolepis-2a  ..........C ........T. ....C..... C.......... ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .........C ........T. ....C..... C.......... ..........
C.armatus-2a    .........C .......... ....C..... C.......... ..........
C.armatus-2b    .........T .......... .......... .......... ..........
C.yaquinae-2a   .........C .......... ....C..... C.......... ..........
C.yaquinae-2b   .........T ........G. .......... .......... ..........

                                                                                                900
C.acrolepis-1   TCATGAAGTG CGACATTGAC ATCCGCAAGG ACCTGTACGC CAACAACGTG
C.acrolepis-2a  .......... ......C... .......... .......... ..........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .......... ......C... .......... .......... ..........
C.armatus-2a    .......... ......C... .......... .......... ..........
C.armatus-2b    .......... ......C... .......... .......... .........T
C.yaquinae-2a   .......... ......C... .......... .......... ..........
C.yaquinae-2b   .......... ......C... .......... .......... ..........

                                                                                                950
C.acrolepis-1   CTCTCCGGTG GTACCACCAT GTACCCTGGT ATTGCTGACC GTATGCAGAA
C.acrolepis-2a  ..G.....C. C........ .......... .......... .C........
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ..G.....C. .C........ .......... ..C....... .C........
C.armatus-2a    ..G.....C. .C........ ......C... ..C....... .C........
C.armatus-2b    ..G....... .......... .......... ..C....... ..........
C.yaquinae-2a   ..G.....C. .C........ ......C... ..C....... .C........
C.yaquinae-2b   ..G....... .......... ......G... ..C..C.... ..........
```

Fig. 11. *continued*

```
                                                                    1000
C.acrolepis-1   GGAGATCACC GCCCTGGCCC CATCCACCAT GAAGATCAAG ATCATTGCTC
C.acrolepis-2a  .......... ........G. .T........ .......... .....C..C.
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   .......... ........G. .T........ .......... .....C..C.
C.armatus-2a    .......... ........G. .T........ .......... .....C..C.
C.armatus-2b    .......... ........A. .C........ .......... ..........
C.yaquinae-2a   .......... ........G. .T........ .......... .....C..C.
C.yaquinae-2b   .........A ........A. .G........ .......... ..........

                                                                    1050
C.acrolepis-1   CCCCCGAGAG GAAGTACTCC GTCTGGATCG GTGGCTCCAT CCTGGCTTCC
C.acrolepis-2a  ....A..... .........T .......... .A........ ......C...
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   ....A..... .........T .......... .A........ ......C...
C.armatus-2a    ....A..... .........T .......... .A........ ......C...
C.armatus-2b    ....A..... .......... .......... .......... ..........
C.yaquinae-2a   ....A..... .........T ..G....... .A........ ......C...
C.yaquinae-2b   ....A..... .......... .......... .......... ..........

                                                                    1100
C.acrolepis-1   CTGTCCACCT TCCAGCAGAT GTGGATCTCC AAGCAGGAGT ACGACGAGGC
C.acrolepis-2a  .......... .......... .......AG. .......... ..........
C.cinereus-1    ........A. .......... .......... .......... ..........
C.cinereus-2a   .......... .......... .......AG. .......... ..........
C.armatus-2a    .......... .......... .......AG. .......... ..........
C.armatus-2b    .......... .......... .......... .......... ..........
C.yaquinae-2a   .......... .......... .......AG. .......... ..........
C.yaquinae-2b   .......... .......... .......... .......... ..........

                                                  STOP              1150
C.acrolepis-1   AGGCCCCAGC ATTGTCCACA GGAAGTGCTT CTAAATCCTC AACATTTGTC
C.acrolepis-2a  C......TC. ..C....... .......... .......AC. C..CCAGCGT
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   C......TC. ..C....... .......... .......AC. C..CCAGCGT
C.armatus-2a    C......TC. ..C....... .......... .......AC. C..CCAGCGT
C.armatus-2b    .......... .......... .......... .......AC. CG.CCAGCGT
C.yaquinae-2a   C......TC. ..C....... .......... .......AC. C..CCAGCAT
C.yaquinae-2b   .......... .......... .......... .......AC. CG.CCAGCGT

                                                                    1200
C.acrolepis-1   TCCATCATTT TCGCCATCAC CATCATCACC ACCTGTCACC AGGAGATCGG
C.acrolepis-2a  CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGA.C
C.cinereus-1    .......... .......... ...T...... .......... ..........
C.cinereus-2a   CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGA.C
C.armatus-2a    CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGA.C
C.armatus-2b    CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGG.C
C.yaquinae-2a   CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGA.C
C.yaquinae-2b   CTGC..TCAG GACA..A.CA ACCGG.GG.A CGAC.G.G.. .CCCAGGA.C

                                                                    1250
C.acrolepis-1   GCGAGGAGGA GGACCACCCC ATGCGGCACA GCGACACCCT GCTGCTCATA
C.acrolepis-2a  CGCCTAGT.G AC.TTTTGTT G.TGTTGTTG TT.TTGTGGA TG..G.AG..
C.cinereus-1    .......... .......... .......... .......... ..........
C.cinereus-2a   CGCCTAGT.G AC.TTTTGTT G.TGTTGTTG TT.TGGATG. .G.AG.AG.T
C.armatus-2a    CGCCTA.T.G AC.TTTTGTT G.TGTTGTTG TT.TTGTGGA TG..G.AG..
C.armatus-2b    CGCCTAGT.G AC.TTTTGTT G.TGTTGTTG TT.TTGTGGA TG..G.AG..
C.yaquinae-2a   CGCCTAGT.G AC.TTTTGTT G.TGTTGTTG TT.TTGTGGA TG..G.AG..
C.yaquinae-2b   CGCCTAGT.G AC.TTTTGTT G.TGTTGTTG TT.TGGATG. .G.AG.AG.T

                                                                    1300
C.acrolepis-1   TGCATTTTTT TTTATGATTC TTGAATCTGC ATATCGTACT GGCTGTCTGT
C.acrolepis-2a  GTTG..G..G ..GTG.TAGT GGA.GAAGTG T.TGGTGGTG T.G.CAACCG
C.cinereus-1    .......... .......... ..AG...... .....TCT.. ..........
C.cinereus-2a   GTTG..G..G .GGTG.TGGA AGAGG.G.TT GGTGGTGTGG TCACCGAA.C
C.armatus-2a    GTTG..G..G ..GTG.TACT GGA.GAAGTG T.TGGTGGTG T.G.CA.C.A
C.armatus-2b    GTTG..G..G .GGTG.TGGT GA...GG..T T.TGG.CTG. .TTG...ACC
C.yaquinae-2a   GTTG..G..G ..GTG.TACT GGA.GAAGTG T.TGGTGGTG T.G.CAACCG
C.yaquinae-2b   GTTG..G.GG .GGTG.TGGA GGAGG.G.TT GGCGGTGTG. TCACCGAA.C
```

Fig. 11. *continued*

```
                                                                    1350
C.acrolepis-1   TGTCTGTGTT GTGCGGAGAA CAAGCTGTGA ACAAACAACA CTGAACCATG
C.acrolepis-2a  AAG.GCAAG. ...TA.C.C. A.CCGGAGCG .GC.G.GGGG GGCG.G..G.
C.cinereus-1    .......... ........G. .......... .........G A.........
C.cinereus-2a   GCAAGTGTG. AGCGCA.ACC GG...GAGC. G.GGGGGG.G AGC.GGG.G.
C.armatus-2a    A.CGCAA..G TGTA.CGC.. .CG.AGCGA. CAGCGGGGGG .GAGCAAGGA
C.armatus-2b    GAAGC.CAAG CGTGTACCGC A.CCGGAGCG .AC.CGGGGG GG.GGAGCA.
C.yaquinae-2a   AAG.GCAAG. ...TA.C.C. A.CCGGA.CG CGGGGGGGGG GG.G.G..G.
C.yaquinae-2b   GCAAGCGTG. AGCGCA.CCG G.GCGA.CAG .AC.CGGCGG GC.G.G..GT

                                                                    1400
C.acrolepis-1   CATCTAACCC CCAAAAAATT AATAAAAAAA TCAAAACAAA AAAAAAAAAA
C.acrolepis-2a  G.GG.C.G.A .GTG..CGCG TGA.G..CTG .AC.T.TG.T T.TTT.TTG.
C.cinereus-1    ....C..... .......... .......... ATC...AC.. C.........
C.cinereus-2a   TCAGC.CGTG AACGCGTGAA G.ACTGT.C. .ATG.TT.TT T.TTG.TT.T
C.armatus-2a    AG..AGCA.G TG..CGCT.G ..AG...CTG .AC.T.ATG. .T.TTT.TTG
C.armatus-2b    TGAGGTCAG. A.GTG..GAA .CA.C..... .A....ACC. CC........
C.yaquinae-2a   G.AG.C.G.A ..TG..CGCG TGA.G..CTG .AC.T.TG.. T.TTT.TTG.
C.yaquinae-2b   G.GG.C.G.A .GTG..G.AA C.AC.....T AA.....C.C C.........

                                                                    1450
C.acrolepis-1   AAAAA
C.acrolepis-2a  TT.TGCAGGC CTACATGGAG GGATTGTTTT GTAAGGACAG GGGCCCGTGG
C.cinereus-1    .....AAAAA A
C.cinereus-2a   GC.GGCCTAC ATGGAGGGAT TGTTTTGTAA GGACAGGGGC CCGTGGCCCC
C.armatus-2a    .TT.TGCAGG CCTACATGGA AGGATGTTTG TTAGAACGGG GGCCCCACGG
C.armatus-2b    .
C.yaquinae-2a   TT.TGCAGGC CTACATGGAG GGATTGTTAG AAAAGGGGCC CACGGCCCCG
C.yaquinae-2b   .

                                                                    1500
C.acrolepis-1
C.acrolepis-2a  CCCCGCAGGC GATGAGTTGG CTGTTGGCTT CGAGGTCGAG GAAAACCCCG
C.cinereus-1
C.cinereus-2a   GCAGGCGATG AGTTGGCTGT TGGCTTCGAG GTCGAGGAAA ACCCCGAACA
C.armatus-2a    CCCCGCAGGA GACGAGTTGG CTGTTGGCTT CGAGGTCGAG GAAAACCCCG
C.armatus-2b
C.yaquinae-2a   CAGGAGACGA GTTGGCTGTT GGCTTCGAGG TCGAGGAAAA CCCCGAACAA
C.yaquinae-2b

                                                                    1550
C.acrolepis-1
C.acrolepis-2a  AACAAAGAAA TGAAAAAGAC CAACAAAAAA AAAAGAACAA AGCAAATAAA
C.cinereus-1
C.cinereus-2a   AAGAAATGAA AAAGACCAAC AAAAAAAAAA AAGAACAAAG CAAATAAAAT
C.armatus-2a    AACAAAGAAA TGAAAAAGAC CAACAAAAAA AAAAAAGAAC AAAGCAAATA
C.armatus-2b
C.yaquinae-2a   AGAAATGAAA AAGACCAACA AAAAAAAAAA AGAACAAAGC AAATAAAATA
C.yaquinae-2b

                                                                    1587
C.acrolepis-1
C.acrolepis-2a  ATAATTTATT TTCTAAAAAA AAAAAAAAAA AAAAAA
C.cinereus-1
C.cinereus-2a   AATTTATTTT CAAAAAAAAA AAAAAAAA
C.armatus-2a    AAATAATTTA TTTTCAAAAA AAAAAAAAAA AAAAAAA
C.armatus-2b
C.yaquinae-2a   ATTTATTTTC AAAAAAAAAA AAAAAAAAAA AA
C.yaquinae-2b
```

Fig. 11. *continued*

Quantification of actin isoforms

To determine the ratio of the α-actin isoforms, quantitative RT-PCR and two-dimensional electrophoresis were performed. These analyses were performed four times using total RNAs and actin preparations isolated from four individuals in each species. The conditions for RT-PCR were the same as described above, except for cycle number. The cycle number within a linear range of PCR amplification was determined to be 25 on the basis of the signal intensities of RT-PCR products with sequential cycles. The primers of 5'-ATTGCTGACCGYATGCAGAA-3' and Not-1 d $(T)_{18}$ amplified approximately 480 bp and 680 bp for actin 1 and actin 2b, respectively. RT-PCR products were subjected to 1.5% agarose gel electrophoresis. Two-dimensional electrophoresis was performed with Multiphor II electrophoresis unit in the pH range of 4.0-7.0 or 5.0-6.0 gel (24cm) and on a 12.5% SDS-PAGE gel (Amersham Bioscience). The ratios of actin 2b to actin 2a, or actin 1 to actin 2a were quantified using a computerized image analysis scanner STORM 860 (Amersham Bioscience).

Phylogenetic analysis

A molecular phylogenetic tree was constructed from the deduced amino acid sequences of actin. The DNADIST and NEIGHBOR programs in the PHYLIP version 3.5 program package (Felsenstein, 1995) were used for neighbor-joining (Saitou and Nei, 1987). Bootstrap analyses with 1000 replicates were performed to examine the confidence of nodes within the resultant topology. The GenBank accession numbers of actin nucleotide sequences used in this study were *C. armatus* 2a (AB086240), *C. armatus* 2b (AB086241), *C. yaquinae* 2a (AB086242), *C. yaquinae* 2b (AB086243), *C. acrolepis* 1 (AB021649), *C. acrolepis* 2a (AB021650), *C. cinereus* 1 (AB021651), *C. cinereus* 2a (AB021652); carp, *Cyprinus carpio* (D50025); medaka, *Oryzias latipes* (D87740); fugu 1, *Fugu rubripes* (U38850), fugu 2 (U38958); goldfish, *Carassius auratus* (D50029); tilapia, *Oreochromis mossambicus* (AB037866); zebrafish, *Danio rerio* (AF180887); chum salmon, *Oncorhynchus keta* (AB032464); atlantic salmon, *Salmo salar* (AF304406); chicken, *Gallus gallus* (K02257); human, *Homo sapiens* (M20543); mouse, *Mus musculus* (M12234); rat, *Rattus norvegicus* (V01218); bovine, *Bos taurus* (U02285). medaka α-actin (D89627) was used as the outgroup gene.

Results

cDNA cloning and deduced amino acid sequences

Two α-actin isoforms were cloned from each *Coryphaenoides* species. The nucleotide sequence was determined for the longest insert including the entire coding region of the positive clones from the respective libraries. The RT-PCR strategy was successful in cloning the entire length of the two α-actin cDNAs from *C. cinereus* (Fig. 11).

Molecular phylogenic analysis based on the coding regions of α-actin gene showed that the two types of isoform each from *C. acrolepis* and *C. cinereus* are categorized as actin 1 and actin 2, respectively, previously reported for pufferfish (Fig. 12). On the other hand, two types each from the two abyssal species were all categorized as actin 2. These categorizations were supported by the comparatively high bootstrap value (72%). Subsequently, the isoform with an identical amino acid sequence to that of actin 2 from the non-abyssal species was designated actin 2a, and the others are designated arm actin 2b and yaq actin 2b from *C. armatus* and *C. yaquinae*, respectively. Consequently, the non-abyssal actin 2 was re-designated actin 2a.

The deduced amino acid sequence of α-actins from four *Coryphaenoides* species contained 337 amino acid residues. Since the N-terminal two amino acids, Met-Cys, are known to be processed after translation (Pollard and Cooper, 1986), α-actin consists of 375 amino acids. The amino acid sequences of α-actin 1 from *C. acrolepis* and α-actin 1 from *C. cinereus*, were identical, as were the amino acid sequences of α-actin 2a from the four species. The amino acid sequences of actin 1 and 2a differ by one amino acid residue at position 155, which is Ala-155 in actin 1 and Ser-155 in actin 2a. The sequence of actin 2b differs from that of actin 2a by two amino acids (Q137K and either V54A or L67P) (Table 4). The X-ray crystallography

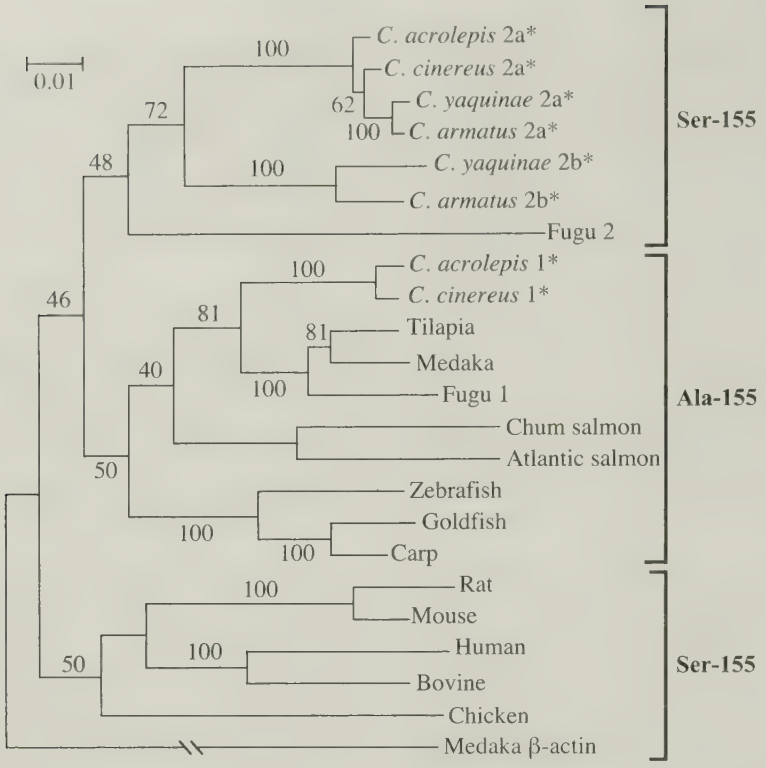

Fig. 12. Molecular phylogentic tree constructed by neighbor-joining method based on nucleotide sequences in the coding regions of actin cDNA from various species. Numbers at internal branches denote the bootstrap percentages of 1000 replicates. The scale indicates the evolutionary distance of the base substitution per site, estimated by the Kimura two-parameter method. The Medaka α-actin gene was used as the outgroup gene. Asterisks indicate the cDNAs that were cloned in this study.

structure of rabbit skeletal muscle α-actin demonstrated that residues 54 and 67 are located in a β-sheet of subdomain 2 (residues 33-69), whereas residues 137 and 155 are located in the Ca^{2+}- and ATP-binding sites, respectively (Kabsch *et al.*, 1990) (Fig. 13).

Northern blot analysis and quantification of actin isoforms

To examine whether these two α-actin isoform mRNAs are expressed in *C. acrolepis*, Northern blot analysis was performed with total RNA that had been extracted from the dorsal skeletal muscle of *C. acrolepis*. Two bands slightly differing on the mobility in the agarose gel were observed (Fig. 14 A). The probes used in the northern blot analysis were derived from 3'-noncoding regions, which considerably differed in nucleotide sequences between the two isoforms. Therefore, the probes were considered to be specific to the respective isoforms. The same result was obtained using total RNA extracted from the dorsal fast skeletal muscle of *C. cinereus*. Two α-actin isoform mRNAs are also expressed in the fast muscle of pufferfish (Venkatesh *et al.*, 1996). The results of Northern blot analysis do not indicate the expression ratio of two isoforms due to the saturation of signal intensities. Therefore, the expression ratio of the α-actin isoform mRNAs were investigated using quantitative RT-PCR. Quantitative RT-PCR was carried out with one primer set, which amplified different length products from the two isoforms about both 480 bp for actin 1 and actin 2b mRNAs, and about 680 bp for actin 2a mRNA showing to the different lengths of their 3' non-coding regions (Fig. 14 B). Direct sequencing confirmed that the RT-PCR products were those of the expected actin isoforms. The results showed that there was differential expression of the two isoforms in each species. The expression ratio of actin 2b to actin 2a mRNA was 4.3 ± 0.18 for *C. yaquinae* and 4.1 ± 0.083 for *C. armatus*, whereas that of actin 1

Table 4. Comparison of the deduced amino acid sequences of actin isoforms from *Coryphaenoides* species and various species

Actin type	Position												
	2	3	54	67	88	137	155	165	228	278	299	354	358
Actin 1	D	E	V	L	T	Q	A	V	A	A	L	S	S
Actin 2a	.	.	.	.	.	.	S	.	.	.	.	.	.
*yaq*actin 2b	.	.	.	P	.	K	S	.	.	.	.	.	.
*arm*actin 2b	.	.	A	.	.	K	S	.	.	.	.	.	.
Pufferfish 1	.	D	.	.	.	.	.	.	.	.	.	.	.
Pufferfish 2	.	D	.	.	S	.	S	I	G	T	.	.	.
Walleye pollack 1	.	.	.	.	.	.	.	.	.	.	.	.	.
Walleye pollack 2	.	.	.	.	.	.	S	.	.	.	.	.	.
Atlantic salmon	.	D	.	.	.	.	S	I	.	.	.	A	T
Carp	.	D	.	.	.	.	.	.	.	.	.	.	T
Zebrafish	.	.	.	.	.	.	.	.	.	.	.	.	.
Medaka	.	.	.	.	.	.	.	.	.	.	.	.	.
Tilapia	.	D	.	.	.	.	.	.	.	.	.	.	.
Chicken	E	D	.	.	.	.	S	I	.	T	M	.	T

"A dot indicates the amino acid residue identical to that in actin 1. Numbers above the sequence indicate the residue position. *yaq*actin 2b and *arm*actin 2b are those cloned from *C. yaquinae* and *C. armatus*, respectively. The GenBank accession numbers for species lists are described in Materials and Methods in Section 2 in Chapter 2. "

to actin 2a was 0.67 ± 0.034 for *C. acrolepis*. The author examined further the ratio at the protein level using two-dimensional electrophoresis. The abundance ratio of actin 2b to actin 2a was 4.8 ± 0.087 for *C. yaquinae* (Fig. 15) and 4.5 ± 0.18 for *C. armatus* (Fig. 15), respectively. The isoforms from *C. acrolepis*, which have the same isoelectric point (5.23) estimated, could not be separated by this electrophoresis method (Fig. 15). These results in the electrophoresis were not affected by dephosphorylation using *Escherichia coli* alkaline phosphatase.

Discussion

One of the most important characteristics of α-actin from deep-sea fish, which enable them to inhabit deep-sea environments, was the extremely small δV associated with actin polymerization, resulting in a lower critical concentration of actin in polymerization at high pressures. Because the small δV was observed even at atmospheric pressure, it is due to actin-actin interactions and not to the relative compressibilities of G- and F-actin. On the basis of the X-ray crystallography structure of actin, however, there are no amino acid substitutions in the regions of actin-actin contact between actin molecules from abyssal and other species.

Two abyssal *Coryphaenoides* species, *C. armatus* and *C. yaquinae*, have three unique amino acid substitutions, V54A or L67P, Q137K and A155S, in comparison with α-actin from non-abyssal *Coryphaenoides* species. The functional significance of these three substitutions in the adaptation to high pressure will be described in Chapter 3.

This study showed that each of non-abyssal species, *C. acrolepis* and *C. cinereus*, have two α-actin isoforms, and that the two isoforms in each species differed by a single amino acid substitution at the 155th residue, Ala-155 in α-actin 1 and Ser-155 in α-actin 2a. The amino acid sequences of α-actin 1 and α-actin 2 from pufferfish (Venkatesh *et al.*, 1996) and walleye pollack have the same substitution of A155S (Table 4). carp, goldfish, tilapia and medaka have a single actin isoform with Ala-155, which has not been found in species except fish. These findings suggest that α-actin 1 with Ala-155 is the common isoform of α-actin in fish. On the other hand, the muscles from rabbit (Collins and Elzinga, 1975), chicken (Chang *et al.*, 1984), mouse (Hu *et al.*, 1986), human (Hanauer *et al.*, 1983) and frog (Stutz and Spohr, 1986) contain only one α-actin with Ser-155. The Ala-155 actin variant is not disadvantageous for freshwater fish such as carp, because they do not experience high pressures as

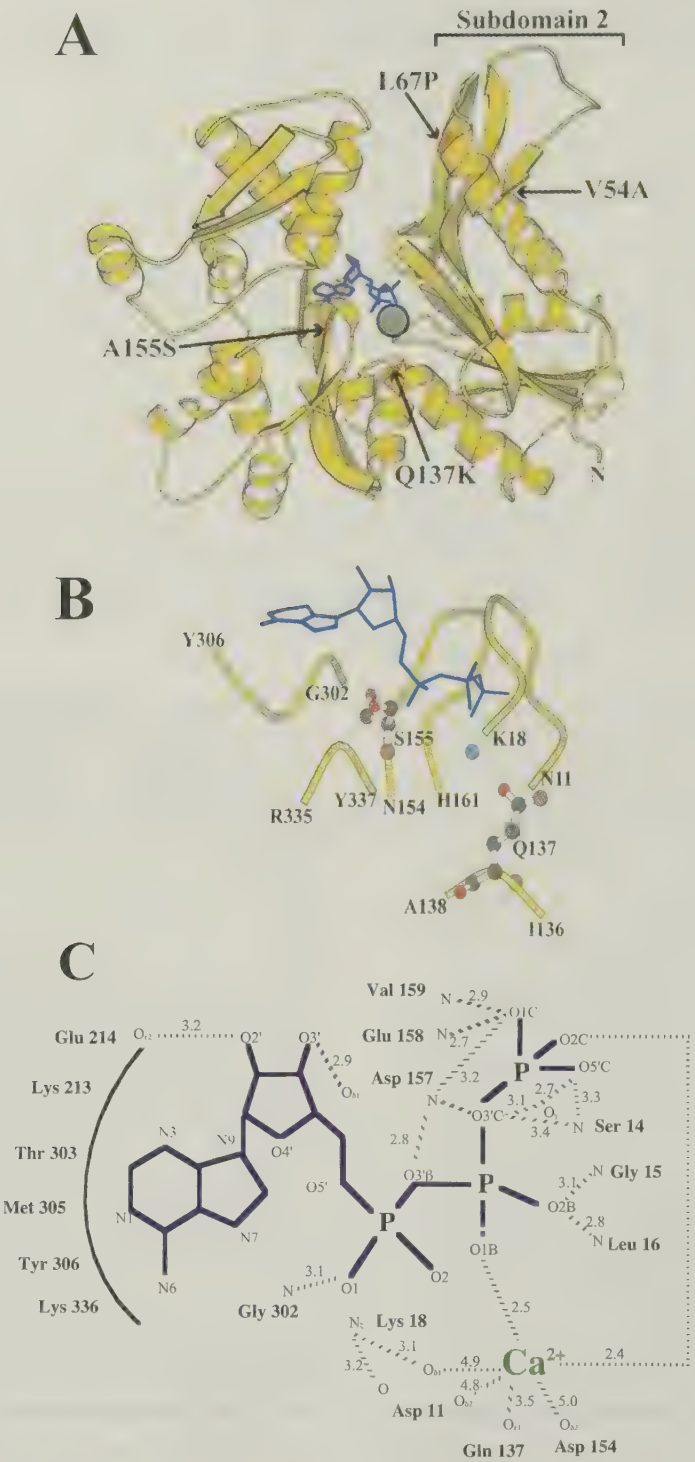

Fig. 13. The structure of the actin bound to ATP and Ca^{2+}. Blue sticks and a greenball represent ATP and Ca^{2+}, respectively. (A) Ribbon drawing of actin (Kabsch *et al.*, 1990). The substitutions found in this study are colored red. (B) Environment of ATP and Ca^{2+} bound to the actin. Gln-137 and Ser-155 are represented by ball and stick. Atoms C, O and N are colored grey, red and purple, respectively. Images A and B are created using MOLSCRIPT (Kraulis, 1991). (C) Schematic drawing for interactions of ATP with actin and Ca^{2+} (Kabsch *et al.*, 1990). Distances between atoms are given in Å. Amino acids of actin are specified near the circular segment in the left part of the drawing from a pocket covering the adenine.

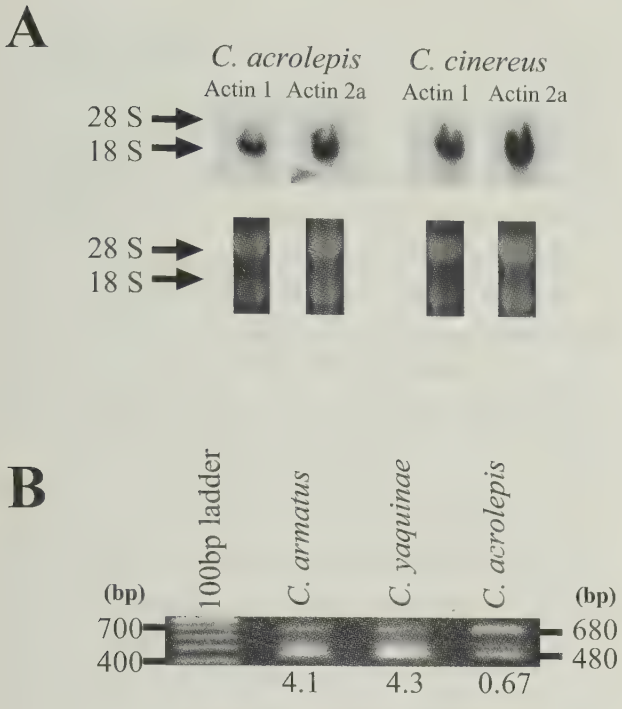

Fig. 14. Northern blot analysis and quantification of actin isoform mRNAs. (A) Northern blot analysis of *C. acrolepis* and *C. cinereus* α-skeletal actin isoform mRNA. The ethidium bromide-stained gels show 28S and 18S rRNA (lower panel), and the separated RNA bands were blotted onto nylon membranes and hybridized with the specific probe for α-skeletal actin 1 and α-skeletal actin 2a (upper panel). (B) RT-PCR analysis for the actin genes from *C. armatus*, *C. yaquinae* and *C. acrolepis*. Numbers indicate the ratios of actin 2b to actin 2a for *C. armatus* and *C. yaquinae*, and actin 1 to actin 2a for *C. acrolepis*.

described in Chapter 3. The molecular phylogenetic tree based on the coding region sequences of α-actin cDNAs clearly showed that actin 2a diverged from actin 1 (Fig. 12). α-actins cloned from freshwater fish so far are the Ala-155 variant. This suggests that the Ala-155 actin is necessary for living in freshwater conditions, and it in marine fish may be a remnant from an ancestral fish. Deep-sea fish, which live under an environment where actin 1 does not completely function as described in Chapter 3, probably have the actin 1 gene but do not express it. The author cannot, however, understand the functional significance of alanine residue at position 155 in actin.

Actin consists of two domains which are historically called large and small domains, although they are nearly in the same size. The small domain contains subdomains 1 [amino acid (a.a.) residues 1-32, 70-144 and 338-372] and 2 (a.a. residues 33-69), while the large domain contains subdomains 3 (a.a. residues 145-180 and 270-337) and 4 (a.a. residues 181-269) (Kabsch *et al.*, 1990) (Fig. 16).

The amino acid at the 155th residue with the Ala to Ser substitution in the two isoforms of α-actin found in the present study is located in subdomain 3. Actin binds ATP by sandwiching β- and γ-phosphates of ATP between two structurally equivalent β-hairpins (residues 11-18 and 154-161), which belong to homologous subdomains 1 and 3. ATP forms a bridge between the small and large domains, and thereby prevents the unfolding of the protein and stabilizes the G-actin structure

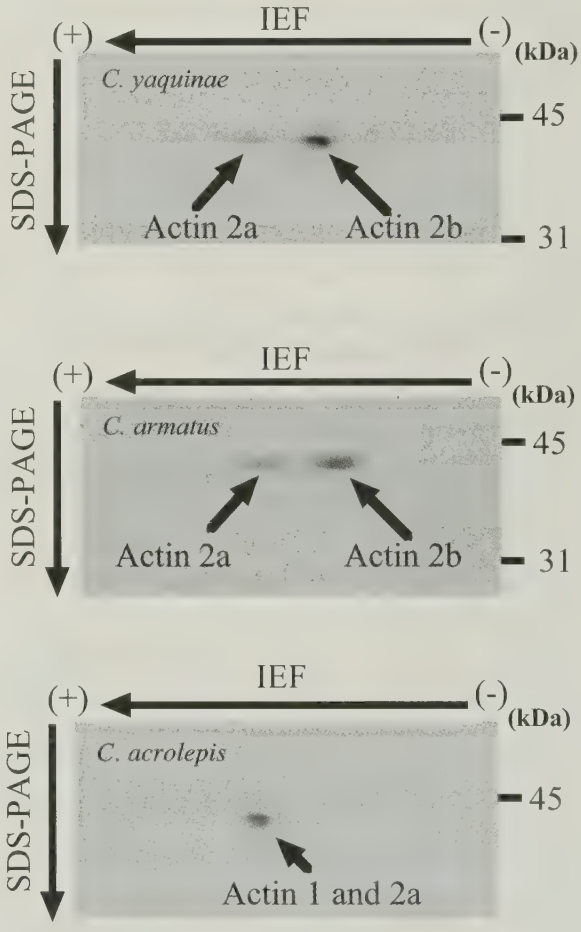

Fig. 15. Quantification of actin isoforms. Two-dimensional electrophoretic patterns of actin from *C. yaquinae*, *C. armatus* and *C. acrolepis*

(Kabsch *et al.*, 1990). ATP is closely associated with a divalent cation in actin, Mg^{2+} in native actin and Ca^{2+} in actin-DNaseI crystals, and regulates the polymerization of actin following hydrolysis of ATP (Carlier, 1991; Kinosian *et al.*, 1991; Reisler, 1993). Ser-14 of the hydroxyl group is one of six residues (Ser-14, Gly-15, Leu-16, Asp-157, Gly-158 and Val-159) involved in stabilizing the position of the phosphates of ATP, and near to the 155th residue in configuration (Kabsch *et al.*, 1990) (Fig. 13 C). The Ser to Ala substitution at the 14th residue results in a 40- to 60-fold decrease in the affinity of actin for ATP and a rapid polymerization of actin than wild type (Chen *et al.*, 1995). Ser to Ala substitution at the 155th found in the present study also might influence the affinity of actin for ATP and/or the divalent cation. Swezey and Somero (1982) reported that the Ser-155 actin from *C. armatus* had a high heat stability. Torigai and Konno (1997) also reported that the heat denaturation of α-actin from carp, rainbow trout and walleye pollack is affected by the concentration of ATP, and that their ATP-bound forms are as stable as rabbit α-actin. In *Saccharomyces cerevisiae*, the Ser to Ala substitution at the 14th leads to a temperature-sensitive phenotype in vivo, and temperature-sensitive polymerization in vitro (Chen and Rubenstein, 1995). These findings suggest that the affinity of actin to ATP influences the heat stability of actin and that α-actin with Ala-155, which is thought to be the common isoform in fish, has weaker affinity for ATP than α-actin with Ser-155 of birds, mammals and amphibians. This is supported by the fact that carp G-actin with Ala-155 and *C. acrolepis* actin with the mixture of Ala-155 and Ser-155 isoforms polymerize more rapidly than chicken and abyssal actins with

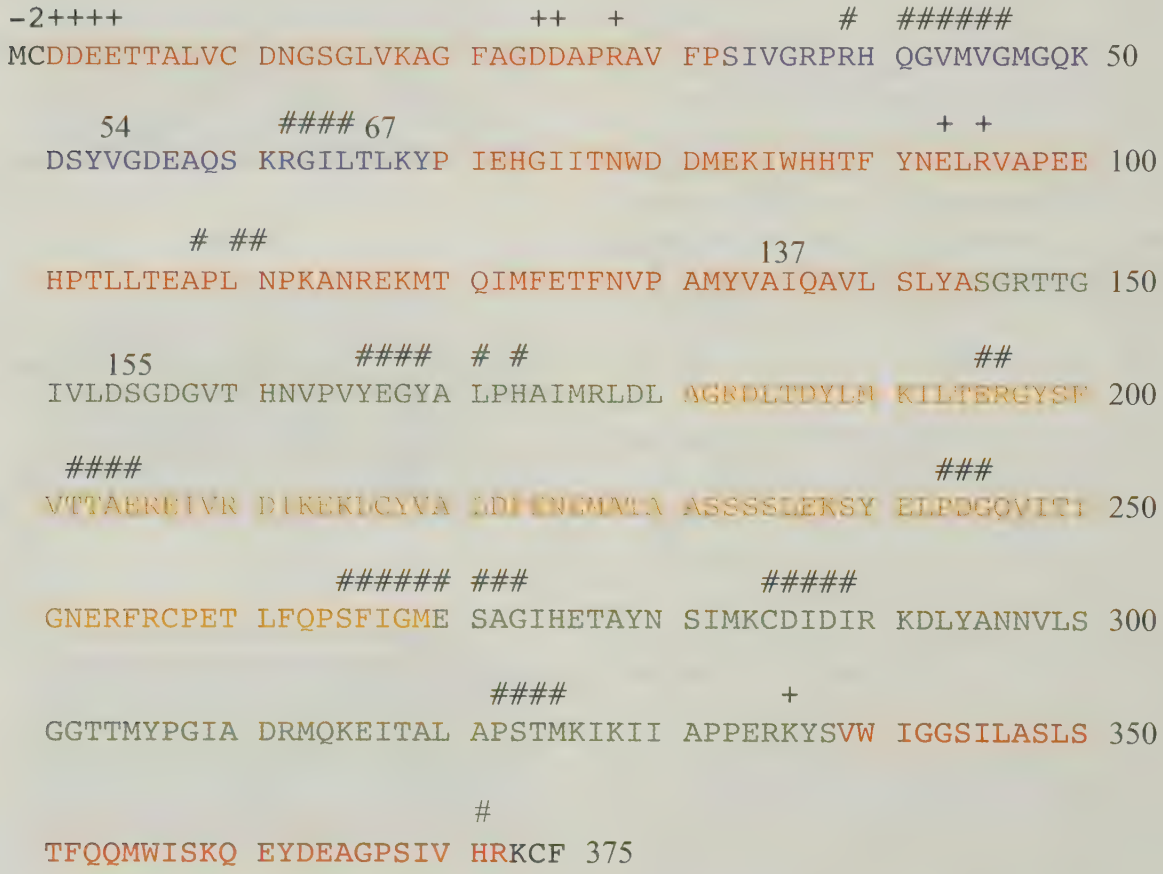
-2++++ ++ + # ######
MCDDEETTALVC DNGSGLVKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK 50
54 #### 67 + +
DSYVGDEAQS KRGILTLKYP IEHGIITNWD DMEKIWHHTF YNELRVAPEE 100
137
HPTLLTEAPL NPKANREKMT QIMFETFNVP AMYVAIQAVL SLYASGRTTG 150
155 #### # # ##
IVLDSGDGVT HNVPVYEGYA LPHAIMRLDL AGRDLTDYLM KILTERGYSF 200
###
VTTAEREIVR DIKEKLCYVA LDFENEMATA ASSSSLEKSY ELPDGQVITI 250
#####
GNERFRCPET LFQPSFIGME SAGIHETAYN SIMKCDIDIR KDLYANNVLS 300
+
GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS 350
#
TFQQMWISKQ EYDEAGPSIV HRKCF 375

Fig. 16. Amino acid sequences of *Coryphaenoides* actin 1. Symbols # and + represent the binding sites to other actin monomers and myosin, respectively. Amino acid residues constructing subdomains 1, 2, 3 and 4 are indicated by red, blue, green and orange characters, respectively.

Ser-155 only (Fig. 10 A).

Chapter 3. Identification of amino acid residues enabling α-actin from the deep-sea fish to adapt to high pressures

In Chapter 2, the author cloned the α-actin cDNAs from two abyssal *Coryphaenoides* species, *C. armatus* and *C. yaquinae*, and identified three unique amino acid substitutions, V54A or L67P, Q137K and A155S, by comparison with α-actin from non-abyssal *Coryphaenoides* species. The X-ray crystallography structure of actin shows that residues 54 and 67 are located in different β-sheets of subdomain 2, and that residues 137 and 155 are located in the Ca^{2+}- and ATP-binding sites, respectively (Kabsch *et al.*, 1990) (Fig. 13).

Actin contains a tightly bound divalent cation (Ca^{2+} or Mg^{2+})in a deep hydrophilic pocket formed by the β- and γ-phosphates of the bound ATP and actin residues Asp-11, Gln-137, and Asp-154 (Kabsch *et al.*, 1990; Estes *et al.*, 1992) (Fig. 13). Although actin has a higher affinity for Ca^{2+} than for Mg^{2+}, much higher cellular concentration of Mg^{2+} indicates that Mg^{2+} will be the main cation bound in vivo (Estes *et al.*, 1992). Actin also binds ATP by sandwiching the β- and γ-phosphates of ATP of between two structurally equivalent β-hairpins consisting of residues 11-18 and 154-161, which locate in homologous subdomains 1 and 3 (Kabsch *et al.*, 1990; Otterbein *et al.*, 2001).

In this chapter, the author investigated the effect of Q137K and A155S substitutions on the binding of actin with divalent cation and nucleotide at various pressures. In addition, he investigated the effect of pressures on the actin structure by measuring the intrinsic tryptophan fluorescence spectrum of G-actin and the DNaseI inhibitation.

Materials and Methods

Material

Samples used were the same in Chapter 2.

Actin

Actin was prepared as described in Chapter 2.

Quin 2 assay

The dissociation rate constant of Ca^{2+} from actin was determined by measuring the increase of fluorescence of 8-amino-2-[(2-amino-5-methylphenoxy) methyl]-6-methoxyquinoline-N,N,N',N'-tetraacetic acid (Quin 2) (Kinosian *et al.*, 1993). Ca^{2+}-actin of 5 μM dialyzed against G-buffer free of $CaCl_2$ was added to 100 μM Quin 2 and 0.2 mM $MgCl_2$ and the changes in fluorescence are measured at excitation and emission wavelengths of 340 and 500 nm, respectively, at 4℃ in a high-pressure cell. The reaction rates were determined by the following relevant thermodynamic equation:

$(\delta \ln k/\delta P) = -\delta V^{\#}/RT$,

where k is rate constant for the reaction, P is pressure, the $V^{\#}$ is activation volume, R is gas constant and T is absolute temperature (Morild, 1981). The apparent binding rate constant and apparent volume change ($\delta V^{\#}$) of the Ca^{2+} dissociation reaction were determined as described previously (Waechter and Engel, 1975; Morild, 1981).

Nucleotide exchange assay

The apparent binding rate constant of $1,N_6$-etheno-ATP (δ-ATP) with actin was determined by displacing bound ATP with a large molar excess of δ-ATP such that the back reaction of ATP binding to actin became negligible (Kinosian *et al.*, 1993). Actin of 9 μM dialyzed against G-buffer free of ATP was converted into Mg^{2+}-G-actin and added to 0.1 mM ε-ATP, and the changes in fluorescence was measured at excitation and emission wavelengths of 340 and 410 nm, respectively, at 4℃ in a high-pressure cell. The apparent binding constant and apparent $\delta V^{\#}$ in the ATP dissociation reaction were determined as described previously (Morild, 1981; Waechter and Engel, 1975).

Intrinsic tryptophan fluorescence

The intrinsic tryptophan fluorescence spectrum of Ca^{2+}-G-actin at 6.25 μM was recorded using a high-pressure cell at excitation and emission wavelengths of 290 and 320-360nm, respectively, at 4℃ (Lehrer and Kerwar, 1972).

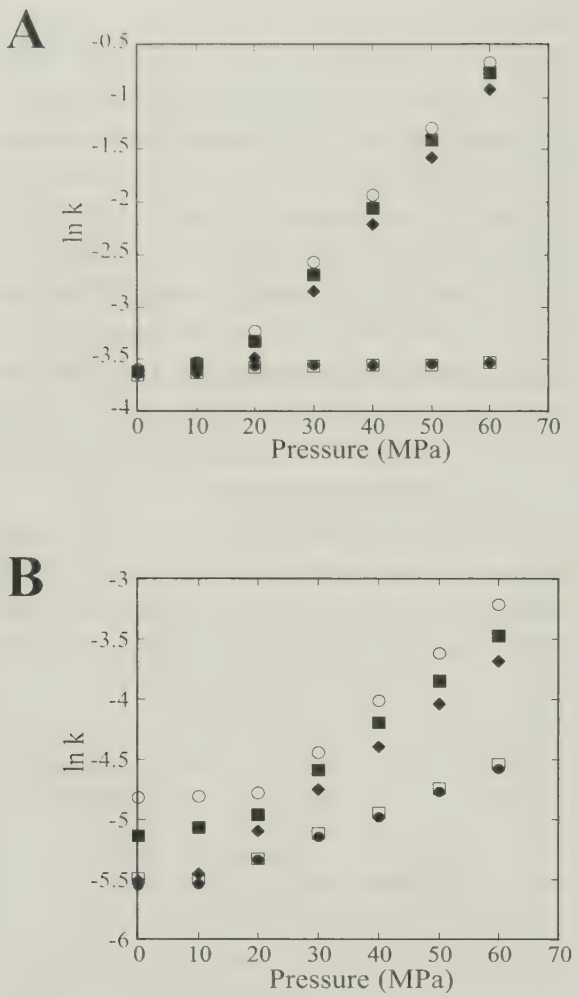

Fig. 17. Logarithmic plots of the dissociation rate constant of Ca^{2+} (A) and ATP (B) in actin at various pressures. Symbols are as follows: abyssal *C. yaquinae* (●); abyssal *C. armatus* (□); chicken (◆); carp (○); non-abyssal *C. acrolepis* (■). Values are means ± S.D. of four independent experiments. Errors for all experiments are too small to show error bars.

DNaseI inhibition assay

The DNaseI inhibition assay was performed as described previously (Blikstad *et al.*, 1978). In brief, DNaseI, either alone or combined with G-actin at various actin/DNaseI ratios, was added to a control or salmon sperm DNA solution containing 100 mM Tris-HCl (pH 7.6), 4 mM $MgSO_4$ and 1.8mM $CaCl_2$ and the change in absorbance at 260 nm (A_{260}) was recorded continuously at 20℃ in a high-pressure cell. DNaseI activity was calculated from the linear part of the plot of the increase in A_{260} versus time.

Results

Sequence analysis showed that actin 2b from abyssal species has lysine at position 137. The other isoform from abyssal species and all actins from non-abyssal species have glutamine at this position, and all actin isoforms from abyssal species have serine at residue 155. Actin binds ATP by sandwiching the β- and γ-phosphates of ATP between two structurally equivalent β-hairpins (residues 11-18 and 154-161), which locate at homologous subdomains 1 and 3 (Kabsch *et al.*, 1990; Otterbein *et al.*, 2001). Actin also contains a tightly bound divalent cation (Ca^{2+} or Mg^{2+}) in a deep hydrophilic pocket formed by the β- and γ- phosphates of the bound ATP and actin residues Asp-11, Gln-137, and Asp-154 (Kabsch *et al.*, 1990; Estes *et al.*, 1992). Although actin has a higher affinity for Ca^{2+} than for Mg^{2+}, much higher cellular concentrations of Mg^{2+} indicate that Mg^{2+} is the main cation bound in vivo (Estes *et al.*, 1992). The author investigated the effect of high pressures on the divalent cation dissociation and nucleotide binding of actin at various pressures (Fig. 17). Surprisingly, both dissociation rate constants of actin from abyssal species were much less affected by high pressures than those of the other actins, which increased rapidly at pressures higher than 20 MPa. The dissociation rate constant of Ca^{2+} in abyssal actins did not differ markedly from those of the other actins at atmospheric pressure, indicating that actins bind to Ca^{2+} with almost the same strength. The differences in the dissociation rate constant of ATP suggests that Ser-155 actin binds ATP more tightly than Ala-155 actin, as pointed out in Chapter 2. The apparent $\delta V^{\#}$ in the Ca^{2+} dissociation reaction at pressures greater than 20 MPa was estimated as -4.27 ± 1.86cm^3 mol^{-1} for abyssal species actins and -154.3 ± 0.583cm^3 mol^{-1} for other actins. The apparent $\delta V^{\#}$ in the ATP dissociation reaction at more than 20 MPa was also determined to be -43.4 ± 0.47cm^3 mol^{-1} or more for abyssal actins, and to be -80.4 ± 1.44, -90.0 ± 1.59 and -84.2 ± 0.995cm^3 mol^{-1} for chicken, carp and non-abyssal actin, respectively. These results indicate that the smaller effect on both

dissociation rate constants of pressure for abyssal actins results from Q137K and the differences in both constants among carp, chicken and non-abyssal species do from A155S.

Effects of pressures on intrinsic tryptophan fluorescence spectrum

Actin contains four tryptophan residues at positions 79, 86, 340 and 356 (Fig. 18), which are all located in subdomain 1. The emission maximum of fluorescing tryptophan residues is 350 nm in a neutral water solution, but shifts to shorter wavelengths in a hydrophobic environment such as in the interior of a folded protein (Kuznetsova *et al.*, 1999). To investigate the effect of pressures on the actin structure, the author measured the intrinsic tryptophan fluorescence of Ca^{2+}-G-actin at various pressures. Although the emission maximum did not shift, the fluorescence intensity of actins from chicken, carp and non-abyssal species began to decrease at only 10 MPa and in particular the decrease for carp actin was larger than that for chicken and non-abyssal actins. By contrast, the fluorescence of abyssal actins did not change even at 60 MPa (Fig. 19). Actin affinity for Ca^{2+} is greater than for Mg^{2+} (Estes *et al.*, 1992), but there were no differences in the fluorescence intensity between the Ca^{2+}- and Mg^{2+}-G-actin forms for all actin species. These results indicate that high pressures change the environment of tryptophan residues in actin, in other words, the structure of actin subdomain 1 including one of β-hairpins that sandwiches the β- and γ-phosphates of ATP and Ca^{2+}-binding sites.

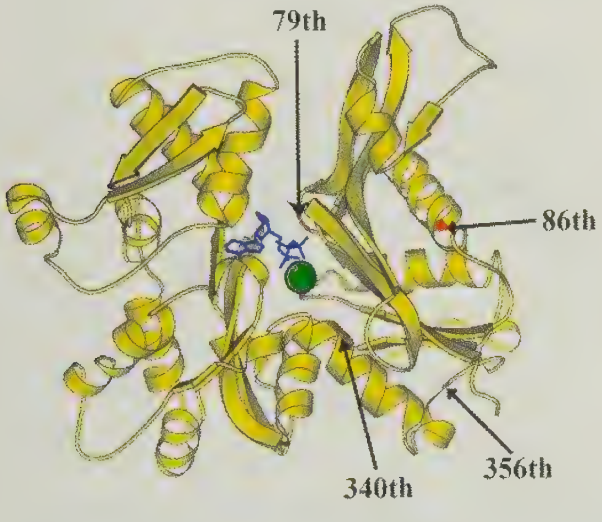

Fig. 18. The positions of tryptophan residues in G-actin. Tryptophan residues at 79, 86, 340, and 356 are colored red. Blue bars indicate ATP, and a green ball does Ca^{2+}.

DNaseI inhibition assay

Actin 2b of the two abyssal species contains either a V54A or an L67P substitution. The X-ray crystallography structure shows that these residues are located in subdomain 2 (Fig. 13 A, 16). In actin-DNaseI interactions, DNaseI primarily contacts the DNaseI-binding loop of residues 40-48 in subdomain 2 and interacts slightly with Thr-203 and Glu-207 in subdomain 4 (residues 181-269) (Kabsch *et al.*, 1990; Khaitlina *et al.*, 1993). To investigate whether these substitutions, V54A and L67P, affect the actin-DNaseI interaction, the author performed a DNaseI inhibition assay at various pressures (Fig. 20). Notably, the author found that abyssal actins inhibited DNaseI activity even at 60MPa, whereas other actins showed little activity at 60MPa. In addition, carp actin was almost inactive at 30MPa. The decrease in DNaseI inhibition activity of actin from 0.1MPa to 60MPa was higher for *C. armatus* actin (23.9%) than for *C. yaquinae* actin (17.2%), in agreement with the fact that *C. yaquinae* lives at higher depths (Endo and Okamura, 1992). These decreases would depend on the denatured state of actin 2a by high pressures, which slightly exists in the muscles of both *C. yaquinae* and *C. armatus* as shown by two-dimensional electrophoresis (Fig. 15). These finding clearly indicate actins from abyssal species were bound to DNaseI even at 60 MPa, suggesting that the substitutions in subdomain 2 of actins from abyssal species are most likely to reduce the increased volumes that occur with the interaction of actin to DNaseI. However, the mechanism underlying the reduction in volume remains unclear.

Discussion

Subdomain 2 consisting of residues 33-69 is probably the most flexible part among all actin

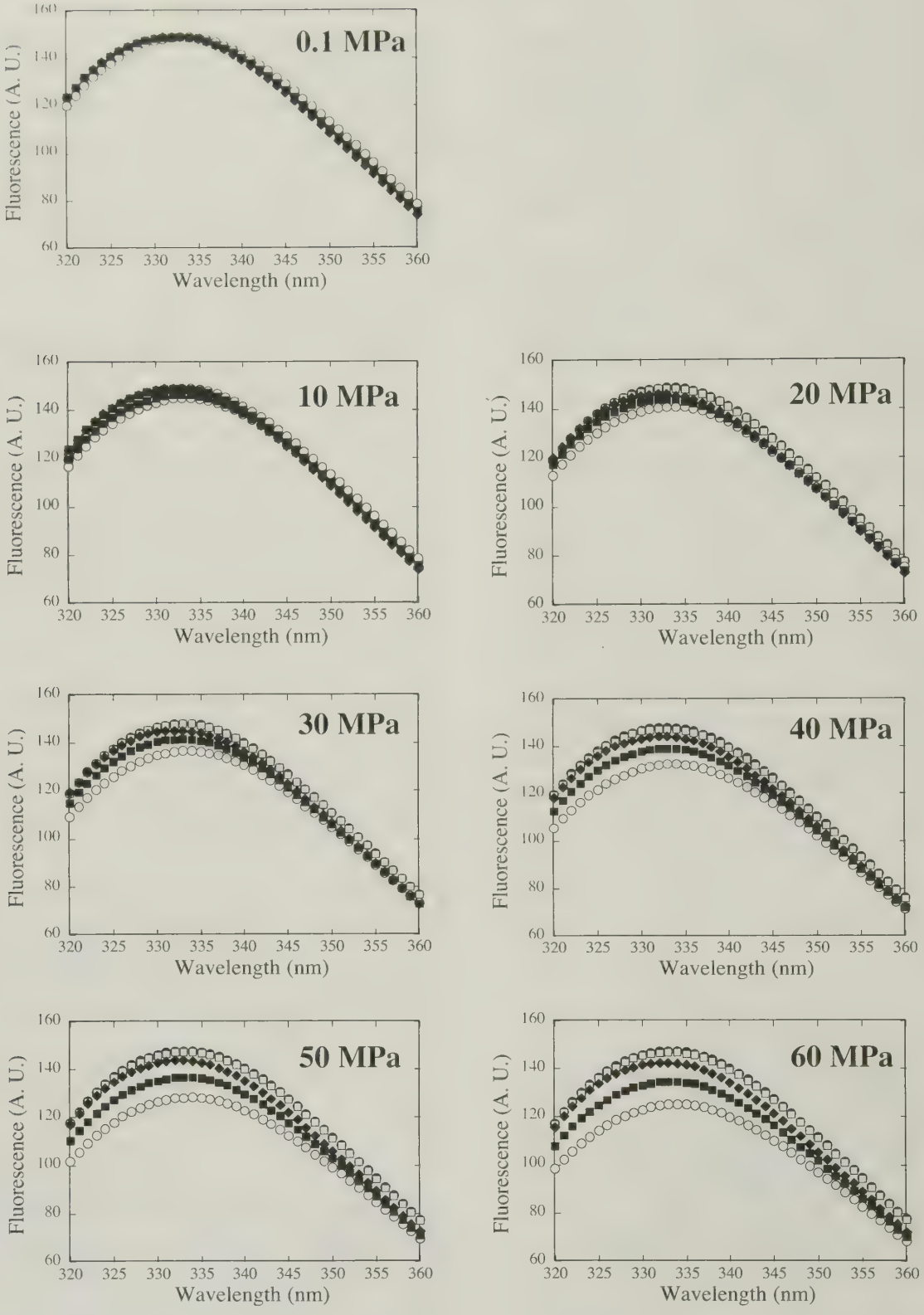

Fig. 19. Effects of pressures from 0.1 MPa to 60 MPa on the intrinsic tryptophan fluorescence spectrum of Ca^{2+}-G-actins. Symbols are as follows: abyssal *C. yaquinae* (●); abyssal *C. armatus* (□); chicken (◆); carp (○); non-abyssal *C. acrolepis* (■).

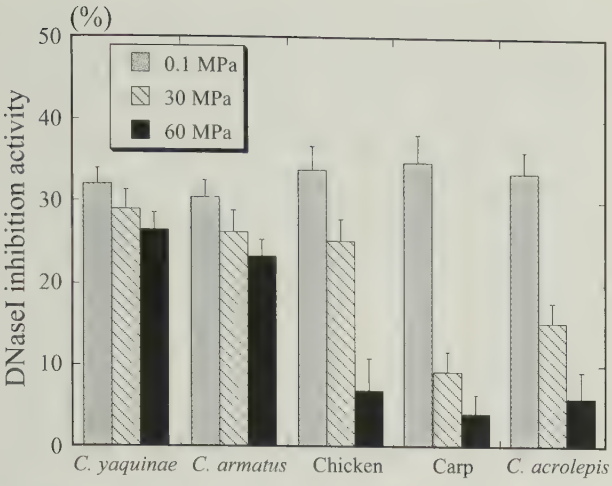

Fig. 20. Effects of pressures on the DNaseI inhibition activity of G-actin. The experiments for each actin were performed at the same ratios of actin/DNaseI at every pressure. Values are means ± S.D. of four independent experiments.

subdomains (Moraczewska *et al.*, 1999) and its conformational change after ATP hydrolysis and Pi release from ADP-Pi during actin polymerization plays a critical role in filament dynamics (Orlova and Egelman, 1993; Belmont *et al.*, 1999). Residues 41-45 in subdomain 2 of one actin molecule interact with two other actin molecules, one at residues 166-169 and the other around residue 375 (Holmes *et al.*, 1990). The author's results show that the DNaseI-binding loop consisting of residues 40-48 in subdomain 2 of abyssal actin can interact with DNaseI even at high pressures, therefore, the author proposes that the abyssal actin also undergoes actin-actin interactions at high pressures without a large increase in volume. Clearly, such a possibility is provided by the V54A or L67P substitution in the β-sheet of subdomain 2 of actin from the abyssal species. Such substitution is known to weaken the formation of β-sheet structure (Levitt, 1978). However, the mechanism of these substitutions reducing δV associated with actin polymerization has not been understood yet.

The dissociation of ATP and Ca^{2+} in particular from actins of abyssal species was less affected by pressure than that of actins from other species. This tolerance to high pressures of actins from abyssal species is due especially to the A155S and Q137K substitutions. These substituted amino acids, which increase the negative value of $\delta V^{\#}$ in both the Ca^{2+} and the ATP dissociation reactions, prevent these reactions from being strongly accelerated by high pressures. Ca^{2+} lies below the bound ATP on the pseudo-twofold axis and is coordinated by four water molecules, which are held in place through interactions with the side chains of Asp-11, Gln-137 and Asp-154, and two O atoms from the β- and γ-phosphates of the bound ATP (Kabsch *et al.*, 1990; Otterbein *et al.*, 2001). Actin, Hsp70 molecular chaperones, hexokinase, and sugar kinases share structural homology and can therefore be considered members of a superfamily (Flaherty *et al.*, 1991; Bork *et al.*, 1992; Hurley, 1996) (Fig. 21). In this superfamily, the amino acid in the equivalent position to Gln-137 found in most actin is usually Asp or Glu, i.e., a negatively charged residue. Surprisingly, actins from abyssal species have positively charged residue Lys at position 137. The Q137K substitution thus changes the coordination of Ca^{2+} with protein. The repulsion between Ca^{2+} and Lys-137 would prevent the Ca^{2+} from being pushed into the bottom of the interdomain cleft by high pressures. On the other hand, another study showed that the rate of ligand entry from solvent to protein interior is increased if the space available to the ligand in the protein pocket is increased by substituting an amino acid residue with a smaller side chain (Gibson *et al.*, 1992). The substitution of amino acids with larger side chains (Gekko and Hasegawa, 1986) such as Q137K and A155S might prevent both Ca^{2+} and ATP dissociation reactions from being accelerated by high pressures. Previous studies have reported that high pressures can slightly affect an apparent Km for substrate in some dehydrogenases from deep-sea fish (Somero, 1990; Siebenaller, 1991; Somero, 1992; Siebenaller and Murray, 1995; Gibbs, 1997). The author's results suggest a mechanism for stabilizing enzyme-substrate interactions under elevated pressures.

The Q137K and A155S substitutions allows that actins other than those of abyssal species to have larger cavities in the protein pocket, owing to the presence of amino acid residues with smaller side chains. The results from Quin 2 and nucleotide exchange assays showed, however, that these

cavities remained unchanged at high pressures. On the other hand, the author's observations here (Fig. 17) and previous studies (Swezey and Somero, 1982) demonstarated that abyssal species actins formed tighter associations with ATP and Ca than other species actins did. The structure of actin is maintained not only by its endogenous weak non-covalent bonds but also by bound ATP and Ca (Kinosian *et al.*, 1993). Thus, tightly bound ATP and Ca^{2+} in abyssal species actins presumably maintain the actin structure from inside, like a pillar. In support of this, carp G-actin was much softer (Fig. 19) and δV of carp actin increased with high pressures (Fig. 10 C), which indicated that for carp actin the coefficient of compressibility for G-actin state was larger than that for F-actin state. Thus, the occurrence of Ala-155 would explain why carp actin was able to polymerize only at 20 MPa or less.

The Ala-155 variant of actin would not be disadvantageous for freshwater fish such as carp, because they do not experience high pressures. However, it would be the disadvantageous for many marine fish. The molecular phylogenetic tree for actin genes clearly showed that actin 2a diverged from actin 1 (Fig. 12). All α-actins cloned from freshwater fish so far are Ala-155 variant, suggesting that Ala-155 actin is necessary for living in freshwater conditions and may be a remnant from an ancester for marine fish. Deep-sea fish, which live in an environment where actin 1 does not function, probably have the actin 1 gene but do not express it. In the superfamily including actin,

Fig. 21. Sequences of structurally conserved regions of actin related superfamily members (Hurley, 1996). The hexokinase sequence is numbered according to the translated gene sequence of yeast hexokinase B. The alignment is based solely on three-dimensional structural equivalence. Only regions that are equivalent structurally in all four proteins are shown. Red residues are either identical in all sequences or have structurally equivalent roles in the nucleotide binding site.

Hsp70 molecular chaperones, hexokinase, and sugar kinases, the amino acid in the position equivalent to 155 in common actin is usually uncharged polar or non-polar amino acid (Flaherty *et al.*, 1991; Bork *et al.*, 1992; Hurley, 1996). Therefore, Ala-155 actin found in many fish would be accepted without any problem in their polarity. However, the author does not understand the full significance of Ala-155 in actin from freshwater fish.

A large portion of the present data was collected from experiments using a mixture of actin isoforms prepared from intact muscle, where the author was unable to separate from each other by biochemical techniques. Although the expression of recombinant proteins in microorganisms is a powerful tool, the system for actin, in particular α-actin, has not been established yet because of various problems, such as the formation of inclusion bodies and low yield of expressed proteins (Buzan *et al.*, 1990). The author is trying to set up expression system of abyssal species actin for future studies.

Chapter 4. General discussion

How do deep-sea fish adapt to high pressures of approximately 60MPa? Many marine biologists have been interested in and made the efforts to unravel this question. Adaptations of Organisms to the environment can be categorized as "tolerance adaptations" which enable an organism to survive in a given environment, and "capacity adaptations" involving regulation of rates of physiological processes, *e.g.* quantitative or qualitative changes of gene expression (Yamashita *et al.*, 1996; Hirayama and Watabe, 1997; Battersby and Moyes, 1998; Hardewig *et al.*, 1999; Kikuchi *et al.*, 1999; Watabe, 2002; Imamura *et al.*, 2003; Itoi *et al.*, 2003). Previous advanced studies have shown that proteins from deep-sea fish functioned even under high hydraulic pressures (Somero, 1990, 1992; Siebenaller, 1991; Siebenaller and Murray, 1995; Gibbs, 1997; Yancey *et al.*, 2001). Therefore, it seems that deep-sea fish selected the tolerance adaptations. While amino acid sequences of proteins, which adapt to high hydraulic pressures, had not been determined, the results in this study indicated, for the first time, amino acid sequences of the unique proteins (Table 4).

Which amino acid substitution contributes to high pressure adaptation of proteins from deep-sea fish, and how does it do? If a reaction proceeds with an increase of volume, increased pressures will inhibit the reaction. The processes, which result in a decrease of volume, will be enhanced by an increase of pressures. Although only reaction with no volume change does not depend on pressures, most biological processes are followed by volume changes. Therefore, proteins from deep-sea fish would change the reaction volume so that their biological reaction is not influenced by high pressures. Thus, two assumptions about the site of amino acid substitution, possibly occurring in proteins from deep-sea fish, were advocated (Somero, 1992). First, substitutions inside proteins and at the protein-water interface bring about alterations in the packing efficiency and hydration volume change, respectively. The latter is that the reaction volume change is produced by amino acid substitution at the ligand binding or subunit contact sites. The present results from deep-sea fish α-actin supported the latter assumption. The first assumption will be supported in other proteins from deep-sea fish, *e.g.* LDH, in the near future.

The present results also showed the differences in mRNA and protein expressions between actin 2a and actin 2b, and between actin 1 and actin 2a (Fig. 14 B, 15). These differences indicate that an actin isoform suitable for adaptation to the environment, in which each fish live, is more abundant at both mRNA and protein levels. These results suggest the capacity adaptations in deep-sea fish by the quantitative change of gene expression.

Recent studies showed that a high concentrating TMAO (trimethlamine oxide) in muscle of deep-sea fish offsets an inhibitory effect of high hydrostatic pressures on biological processes (Yancey and Siebenaller, 1999; Yancey *et al.*, 2001; Yancey *et al.*, 2002). Other studies also indicated that high pressures have the membrane-ordering effects on deep-sea organisms, which change membrane lipid composition and fluidity, by increasing the proportion of unsaturated fatty acids (Phleger and Laub, 1975; Cossins and Macdonald, 1984, 1986, 1989). The membrane lipids alone are not, however, related

to high pressure adaptation in the case of sodium pump (Gibbs and Somero, 1990; Gibbs, 1995). As the present results and those studies showed, deep-sea fish advanced into the largest space among the ocean (Fig. 1) not only by amino acid substitution in proteins but also by the changes in concentration of low molecules such as TMAO and membrane composition. Thus, the advancement into deep-sea would be result of integration of many contingencies which happened according to the neutral theory of molecular evolution (Kimura, 1968). The separation between abyssal and non-abyssal *Coryphaenoides* is estimated to have occured approximately 3.2-7.6 million years ago (Fig. 8).

There are a number of actin-binding proteins (Pollard and Cooper, 1986) and, as a result, actin is one of the most conserved proteins with only limited variations. The present results have shown that a novel function of a protein that enables species to adapt to a new environment can be achieved by a very few amino acid substitutions in key functional positions. Moreover, the molecular phylogenetic tree based on α-actin coding region showed that actin 2a diverged from actin 1 and actin 2b did from actin 2a, again by a very few amino acid substitutions. Freshwater fish had only Ala-155 actin, i.e., actin 1 (Table 4, Fig. 12). Thus, when teleosts advanced from freshwater to sea and continuously did from surface to abyssal zone, they would have duplicated α-actin gene each time. Such observations are consistent with the Perutz's theory of protein speciation (Perutz, 1983), the theory of gene duplication (Ohno, 1970) and the prediction from the molecular phylogenetic tree based on mitochondrial DNA analysis (Fig. 6, 7, 8).

Low temperature (1-4℃) is also one of physical factors characteristic of the deep-sea. A_4-lactate dehydrogenase (A_4-LDH; 1.1.1.27) of Antarctic notothenioid fish has been well known to adapt to extreme cold temperatures (Somero, 1995), and those amino acid sequences were recently determined (Fields and Somero, 1998). A_4-LDH of deep-sea fish has also been known to adapt to high pressures and low temperatures (Somero, 1990; Siebenaller, 1991; Somero, 1992; Siebenaller and Murray, 1995; Gibbs, 1997). High pressures together with low temperatures can slightly affect an apparent Km of substrate in some dehydrogenases from deep-sea fish. It is of interest whether deep-sea fish have unique substitutions similar to those found in A_4-LDH of Antarctic notothenioid fish.

It has been considered that new species occur when gene flow between different populations is interrupted (France and Kocher, 1996). High hydraulic pressures are considered to accelerate the speciation process as a barrier of gene flow even in the absence of absolute geographic isolation, since hydraulic pressures have various effects on biochemical and physiological processes (Siebenaller and Somero, 1978, 1989; Siebenaller, 1991; Childress and Thuesen, 1995; Gibbs, 1995; Siebenaller and Murray, 1995). The present study indicates that differences in the adaptation of α-actin to hydraulic pressures produced the speciation between abyssal and non-abyssal species. Considering that the molecular phylogenetic trees by mtDNA analysis do not clearly define the taxonomic relationships of non-abyssal species, factors other than those participating in biochemical processes, *e.g.* food habit, are most likely to be concerned in the speciation of non-abyssal fish in the upper continental slope.

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和文要旨

大容量試料を用いた改良測定法による海水中有機スズ化合物の定量及び海水中での存在状態の考察

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既存の測定法を改変し大容量の水試料を用いることで，海水中有機スズ化合物（OTs）をより低濃度まで測定することに成功した。広島湾や呉港で採取した海水や浮遊懸濁物質（SS）についてOTsを測定し，これらの結果から海水中OTs存在割合を計算すると溶存態に0.51-0.81，SSに0.19-0.49となり，多くのOTsは溶存態として存在することが示された。また，表層水中OTs濃度とSS重量の間には正の相関があることが示された。

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生物起源粒子中のどこにカドミウムは存在するか？

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沖縄県石垣島近海の亜熱帯海域表層における生物起源粒子（炭酸カルシウムの殻及び有機物）中のカドミウムを調べた。その結果，生物起源カドミウムの多くが有機物区分に存在していた。また，過酸化水素水による加熱抽出時における炭酸カルシウム殻の溶解の可能性等が考えられ，今後の検討課題として残された。

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太平洋戦争以前および終戦直後の日本のまぐろ漁業データ探索

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太平洋戦争以前およびその直後における日本のまぐろ漁業の情報として，どのような科学的データが残されているのかを探索して本稿にまとめた。結果として，科学的な信用に値し，しかも系統だった戦前の漁業データとしては，現在で言うところの農林水産省の「農林統計」と各県の水産試験場調査船によるはえ縄と竿釣データが利用可能な情報であると考えられる。

農林統計においては年間の魚種別漁獲量統計が明治27年（1894年）から，漁業種類別の漁船数が1905年から編纂されている。かつお類の漁獲量は，沿岸漁業が主体であった1914年以前には３～５万トンであったが，その後の沖合漁業の発達により，1915～1935年には６～８万トン，1936～1940年には10万トン以上へと増加している。まぐろ類については，1918年頃までは沿岸漁業による漁獲が主体であり，総漁獲量も２万トンを超えることはなかったが，漁船の機械化による沖合でのはえ縄漁業および流し網漁業の発達に伴い漁獲は急増し，1929年には６万トンを超えている。

水産試験場調査船の操業データについては，1933年から1942年までの10年間に，はえ縄で計5,302操業，竿釣で計3,315操業が記録されており，操業日および位置が特定されるかつお・まぐろ漁業の努力量・漁獲量データとしては最も古い情報であると思われる。はえ縄操業に関しては，1934年から1937年頃までは日本近海，北緯20度以北の北西太平洋におけるビンナガを主対象とした操業が主であり，西部太平洋熱帯域では操業は行われていなかった。1938年以降，西部太平洋の北緯20度から赤道付近に操業が広がり始め，1940年以降では東経155度以東の北西太平洋における操業は姿を消し，主漁場は北緯10度から赤道付近までの西部太平洋（ヤップ，パラオ周辺とミクロネシア周辺）に移行してキハダを主対象とする操業が行われた。また，操業時刻のデータから，当時すでにメカジキやさめ類を漁獲するために夜縄が用いられていたことが示唆される。かつお類を主に漁獲する竿釣操業については，データが収録された全ての期間において操業は日本近海で行われており，はえ縄のように南方への漁場の広がりは認められない。資源評価におけるこれらの情報の有用性については，魚種によっても異なるであろうが，戦後のデータとの比較を通して今後検討していくことが必要であろう。

終戦直後から主要な水揚げ港における入港船の航海日誌を書き写すことによって，かつお・まぐろ漁業のデータ収集が開始された。しかし，マッカーサーラインが撤廃された1952年以降の操業データから編纂が始められたため，1951年までのデータは手書き用紙の状態で保存されており，そのデータ編纂が望まれる。

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深海魚α-アクチンの高水圧適応の分子機構に関する研究

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ある種の深海魚は水圧が600 気圧にも及ぶ深海に生息している。その高水圧適応機構については古くから関心が持たれてきたが，タンパク質構造と高水圧下での機能との関係を明らかにした報告はこれまでにない。本研究では，深海性ソコダラ類のヨロイダラ *Coryphaenoides armatus* およびシンカイヨロイダラ *C. yaquinae* の骨格筋α-アクチンを対象に高水圧適応機構を分子レベルで解明した。本論文の構成は次の通りである。第１章では，ホカケダラ属（*Coryphaenoides*）の分子系統樹の作成を行った。ホカケダラ属のソコダラ類は，浅海から深海と幅広い水深に生息する同属種が存在することから，深海魚の特性を探る様々な研究に用いられている。比較生化学研究を行う対象魚を選択することを目的として，ミ

トコンドリア12S rRNA および cytochrome oxidase subunit I（COI）遺伝子の部分配列を決定し，本属の分子系統樹を作成した。本系統樹から深海性および浅海性ソコダラ類がホカケダラ属の進化の初期の段階で分岐したことが示された。得られた系統樹を参考に，深海性ソコダラ類であるヨロイダラおよびシンカイヨロイダラの比較対象魚として，浅海性ソコダラ類からイバラヒゲおよびカラフトソコダラを選択した。第2章では，深海性ソコダラ類α-アクチンの高水圧下での性状変化を検討した。深海性ソコダラ類2種，浅海性のソコダラ類イバラヒゲ，淡水魚のコイおよび陸上動物のニワトリの骨格筋からα-アクチンを精製し，高水圧下で重合に要する時間，臨界濃度及び重合に伴う体積増加量を調べた。いずれの分析においても深海性ソコダラ類のα-アクチンは，大気圧下とほぼ変わらぬ性状を示した。第3章では，深海性ソコダラ類α-アクチンの cDNA クローニング及び高水圧適応に必須のアミノ酸の同定を行った。深海性ソコダラ類と浅海性ソコダラ類の骨格筋からα-アクチンの cDNA クローニングを行った結果，それぞれ2タイプずつのα-アクチン cDNA が単離された。ノザンブロット解析，定量 RT-PCR 法及び2次元電気泳動法から，これらα-アクチン mRNA 及びタンパク質のいずれも骨格筋中に存在していること，その存在量は高水圧に適応しているタイプがいずれの形態でも多く存在していることが示された。演繹アミノ酸配列において，深海性ソコダラ類に特異的なアクチンのタイプは，浅海性ソコダラ類に特異的なタイプと比べて Q137K，A155S および V54A または L67P の計3カ所にアミノ酸置換を示した。幾つかの生化学的実験から，Q137K および A155S の両置換はα-アクチン分子内に Ca^{2+} と ATP が高圧によって押し込まれるのを防ぎ，深海性ソコダラ類に高水圧適応を付与していることが示唆された。さらに deoxyribonuclease I（DNase I）とアクチンの結合実験から，深海性ソコダラ類のα-アクチンが高水圧下で重合するためには，V54A または L67P の置換が重要であることが推測された。第4章では，高水圧適応と低水温適応との関連などを含む総合的考察を行った。

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